THE EFFECT OF NUTRITION ON THE PERIPARTURIENT PARASITE STATUS OF SHEEP

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ABSTRACT

Four trials were conducted to investigate the role of nutrition in the periparturient breakdown of resistance to gastrointestinal parasitism in mature sheep. In the first trial pregnant ewes were housed seven weeks prior to parturition and fed either to gain (HE) or lose (LE) maternal body weight. From five weeks prior to parturition ewes were trickle infected with 4,000 *T.circumcincta* larvae day^{-1}. Faecal egg counts were monitored throughout the infection period. Ewes were slaughtered either at partum (S0) or six (S6) or nine (S9) weeks post partum for determination of worm burden. S6 and S9 groups received an additional challenge infection of 25,000 *T.circumcincta* larvae, 21 days prior to slaughter. Live weight gain was significantly greater in HE than LE ewes *viz.* 15.5 kg and 6.0 kg, respectively. Despite this difference mean faecal egg counts were significantly higher in LE sheep only in the week preceding parturition *viz.* 23 and 252 eggs g^{-1} faeces, for HE and LE groups, respectively. Nutrient supply had no effect on worm burdens at any of the three slaughter times.

Trial 2 examined the combined effects of current nutrient intake and level of larval challenge on the magnitude of the periparturient breakdown. Ewes were housed nine weeks prior to parturition. One group was fed above the recommended energy requirements (H10, n=12) and trickle infected with 10,000 *T. circumcincta* larvae day^{-1} from 8 weeks before lambing. Groups L5, L10 and L20 (n=12) were fed below their recommended energy requirement and trickle infected with 5,000, 10,000 and 20,000 *T. circumcincta* larvae day^{-1}, respectively. Faecal egg counts were recorded throughout. Ewes were slaughtered at parturition. Again, despite significant differences in live weight gain and body condition score between H and L groups, there was no effect of nutritional treatment on parasite status. Similarly, larval challenge did not affect parasite status.

Results from Trials 1 and 2 thus questioned the role of dietary energy intake in maintaining resistance to gastrointestinal parasitism. The latter study also demonstrated the unimportance of larval intake on the periparturient breakdown.

Trial 3 was undertaken to assess the relative importance of metabolisable energy and metabolisable protein supply. Utilising a 2x2 factorial design for energy (E) and protein (P) single and twin bearing ewes were fed either a basal ration (adequate E and P) or at a supplemented level (E and P in excess of requirements). Energy levels were altered by varying the hay:grain ratio of the diet. Protein supplementation was achieved by inclusion of 75 g fishmeal kg^{-1} dry matter in rations. Ewes were trickle infected with 10,000 *T. circumcincta* and 7,000 *T. colubriformis* larvae day^{-1} from seven weeks before lambing. Larval dosing ceased at parturition and the ewes were slaughtered three weeks later. There was no effect of energy supplementation on worm burdens and only a short term effect on faecal egg counts in the week before lambing. Protein supplementation significantly reduced faecal egg counts from 21 days before lambing and throughout the remainder of the trial. Worm burdens in protein supplemented sheep were significantly lower than those on the basal protein ration *viz.* 1,540 worms compared with 12,020. Single bearing sheep had significantly lower worm burdens than twin bearing sheep (2,300 compared with 8,100). Worm burdens comprised mainly *Teladorsagia* spp. Less than 4% of total worm burdens consisted of *Trichostrongylus* spp. There was no effect of nutritional treatment on lymphocyte stimulation in response to various parasite antigens and mitogens. Similarly, inhibition of larval migration in response to small intestinal mucus was unaffected by nutritional treatment. Results from Trial 3 indicated the relative importance of protein supply over energy intake in periparturient resistance to gastrointestinal parasitism.

A fourth and final trial was undertaken to determine the appropriate threshold level of protein supplementation required to achieve reductions in the extent of the periparturient breakdown. Three treatment groups of twin bearing ewes (n=10) were established ten weeks prior to parturition. Diets were designed to provide approximately the same level of metabolisable energy but to differ in their metabolisable protein provision. This was achieved by the inclusion of 0, 100 and 200 g of fishmeal

kg^{-1} dry matter in the diet of groups F0, F10 and F20, respectively. The ewes were housed eight weeks prior to parturition and trickle infected daily with 10,000 *T. circumcincta* and 7,000 *T. colubriformis* larvae, during the 42 days before parturition. Infective dosing ceased at parturition. Eleven days later five animals from each group were given a further single challenge infection of a 25,000 *T. circumcincta* and 17,500 *T. colubriformis* infective larvae. All ewes were slaughtered ten days later. Faecal egg counts of the F20 group remained below 100 eggs g^{-1} of faeces throughout the course of the experiment. Faecal egg counts immediately prior to slaughter were significantly lower in sheep which had received the post partum challenge infection. Worm burdens of both species decreased with increasing protein supply. Sheep which had received the post partum challenge infection had significantly higher numbers of L4 larvae than those trickle infected only. Establishment of *Trichostrongylus* spp. from the post partum challenge infection was significantly higher in F0 than F20 sheep. Establishment rates were calculated to be 15.2, 9.4 and 5.9% in *Teladorsagia* spp. and 9.3, 3.4 and 0.9% in *Trichostrongylus* spp., for groups F0, F10 and F20, respectively. These results confirmed that protein supplementation can moderate the periparturient breakdown. Results from the post partum challenge infection provided evidence that the effects of protein on worm burdens may have occurred at the establishment stage against incoming larvae.

Results from this study indicate that protein supplementation may be an important component in strategies aimed at moderating the deleterious effects of the periparturient breakdown. Energy supplementation, ewe body condition and larval challenge appear to be less important but may impact under more intense conditions. The means by which protein supply affects resistance to parasitism in the mature ewe deserves further investigation.

Key words: gastrointestinal parasitism; periparturient breakdown; metabolisable energy; metabolisable protein; host resistance.

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Chapter 1

Introduction

The control of gastrointestinal (GI) parasitism in ruminant animal production systems has, in the past, relied heavily on anthelmintic chemotherapy. In recent years however, the emergence of anthelmintic resistance and growing consumer concern over possible residues and ecotoxicity has raised awareness of the need to develop alternative control programmes of a more sustainable nature. A complete abandonment of chemotherapeutic control is not advocated as this is often an essential component of intensive sheep production. Rather, an integrated approach, which reduces the reliance on anthelmintics, while enhancing strategies within farming systems, which minimise parasite burdens faced by grazing stock, may be more appropriate. One such approach could be to target the source of the infection.

Young lambs appear to have little innate immunity to parasitic infection and due to the nature of the disease, parasitic gastroenteritis, require considerable prophylactic treatment to achieve their productive potential. There is evidence to indicate that the main source of infection, to which these naive lambs are exposed, originates from their dams.

Resistance to parasitism is acquired with age and exposure to infection. By the time the animal reaches it's mature breeding age it is generally resistant to the effects of GI parasitism. However, it is well recognised that during the periparturient period, ewes experience a temporary relaxation of resistance to nematode infection, the consequence of which is a rise in the output of nematode eggs in the faeces of affected animals. The larvae developing from these eggs, thus provide a reservoir of infection for young lambs.

The cause of the periparturient breakdown in resistance is unclear but was summarised by Barger (1993) as being variously attributed to poor nutrition, stress, lack of antigenic stimulation and hormonal suppression. To date, attempts to identify the precise cause have been equivocal and further investigations are warranted.

The mechanisms involved in providing resistance to parasitism appear complex but there is evidence that host nutrition may play an important role in the development and maintenance of resistance to infection. In the young animal, facing intense nutrient requirements for growth, it appears that protein supplementation can enhance the rate of acquisition of resistance. In the mature animal, nutrient requirements are similarly intense during the periparturient period and this may impact on the immunological status of the animal.

This study was undertaken to elucidate the role of nutrition in the periparturient parasite status of mature sheep.

Chapter 2

Review of Literature

2.1 The periparturient breakdown in resistance to gastrointestinal parasitism

The control of gastrointestinal (GI) parasitism in grazing sheep has historically relied on chemical drench treatment, using a range of anthelmintic compounds. In the last 40 years these anthelmintics have been an essential component of sheep production systems, often with greater than 95% efficacy against parasite burdens (Sykes *et al.*, 1992). In recent years however the emergence of isolates of parasites resistant to these chemicals (Watson and Hosking, 1990; Jackson, 1993; Rolfe, 1997), has raised concerns over the sustainability of animal production systems so reliant on anthelmintic treatment (Waller, 1997). In addition to this, the likelihood of growing consumer concern over chemical residues in sheep products and the increasing costs involved in controlling GI parasitism, has lead many workers to investigate alternative means of controlling GI parasitism (Barger and Southcott, 1978; Brunsdon, 1980; Waller, 1993; McKellar, 1997).

One approach has been to study the epidemiology of parasites within production systems in order that action may be taken to reduce exposure of animals to large infestations (Sykes *et al.*, 1992). Young, growing lambs, in their first year of life are highly susceptible to parasite infection and, as a consequence, require more frequent anthelmintic treatment than other classes of stock. The reasons for this are two-fold. Firstly, the immunocompetancy of young animals to nematode infection is considerably lower than that of mature stock and secondly, young stock are often exposed to very high levels of infective larvae while grazing with their dams. If steps are to be taken to reduce the frequency of anthelmintic usage, then it would seem appropriate to address these two issues. As the

acquisition of resistance appears to be age dependent (Gibson and Parfitt, 1972; Smith *et al.*, 1985; Lloyd and Soulsby, 1987; Kambara *et al.*, 1993), it may be more prudent to aim to reduce levels of pasture contamination *viz.* by targeting the source of the infection. It is generally acknowledged that the mature breeding ewe is a major source of infective larvae to which naive young stock are exposed (Brunsdon, 1966; Heath and Michel, 1969; Boag and Thomas, 1971; Reid and Armour, 1975). A temporary relaxation of the ewe's own resistance to parasites around parturition results in an increase in faecal nematode egg output - the larvae of which contribute to the pool of infection on pasture to which young stock are exposed. The precise cause of this relaxation of resistance has yet to be identified.

2.2 Gastrointestinal parasitism of ruminants

All grazing animals are continually exposed to parasitic worms which they ingest with the pasture they consume. Under the most natural conditions, where the animal (host) grazes without restriction, the host/parasite relationship favours the parasite. The detrimental effects of parasitism on the host are limited in their extent, as it is not in the `interest' of the parasite to kill the host. Domestication and intensive grazing systems mean that the host is restricted in terms of grazing area and pasture selection and the natural balance between host and parasite is disrupted. As a consequence, the animal will face greater levels of parasite challenge, which may have severe, detrimental effects on health and may, in extreme cases result in the death of the host.

Livestock systems aim to optimise production from animals in the form of lean tissue, milk, wool etc. Parasitic infection acts to reduce this productivity and consequently, much research effort has gone into the investigation of possible means to overcome these losses.

The life cycle of gastrointestinal parasites

There are many parasites capable of infecting grazing livestock but the nematode helminths are among the most economically damaging to production systems (Charleston, 1982). Many of these share a common life cycle. The adult parasites live within the GI tract of the host. Different species of nematode have different sites of infection (Table 2.1). The adults reproduce and the female lays eggs which are passed out of the host in the faeces onto the pasture (Figure 2.1). The eggs hatch within the faecal pellet, under favourable environmental conditions *viz.* temperature and moisture. Still within the faecal pellet, the larvae undergo a series of moults through L1 to L3 developmental stages (Figure 2.1.). On reaching the L3 stage the larvae migrate from the faeces onto pasture. It is at this L3 stage that the larvae become infective and now require to be ingested by a suitable host animal to reach maturity. The L3 larvae remain on the lower 5 cm of the pasture sward and in some cases in the soil profile, protected by a tough outer skin (cuticle).

Once inside the host the larvae pass through the rumen of the animal and shed this outer skin (exsheath) on entry to their specific site of infection. At this stage the larvae can either continue development through L4 and L5 stages to reach adulthood or, in some species, may undergo a period of arrested or inhibited development and remain dormant within the mucosa of the host. It is believed that inhibition is a survival mechanism and enables the parasite to remain dormant while the host is immunologically resistant to infection or where challenge levels are so high as to compromise parasite establishment (Soulsby, 1972). Other workers have hypothesised that inhibition may reflect seasonal effects on the physiology of either the host (Gibbs, 1967; Connan, 1968) or on the larva (Anderson *et al.*, 1965; Armour *et al.*, 1969; Reid and Armour, 1972). The precise mechanism by which inhibition is triggered has yet to be determined but it's occurrence enables the parasite to survive periods which could potentially interfere with their development to adulthood.

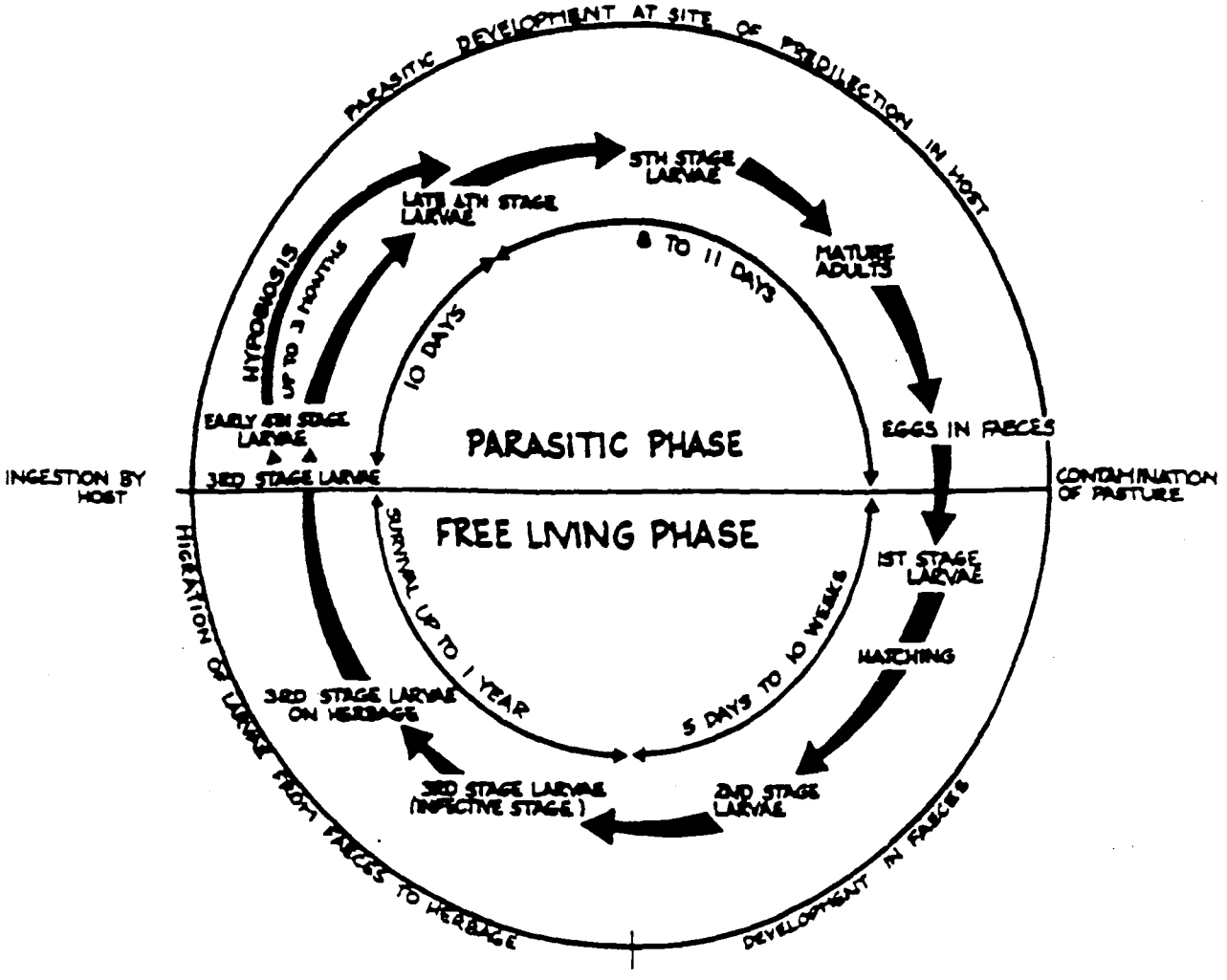

Figure 2.1 Generalised life cycle of Trichostrongyle nematode (Adapted from Brunsdon, 1982)

Table 2.1 Gastrointestinal parasites of sheep in New Zealand: site of infection and their importance

Level of importance	Major	Secondary or occasional	Little or zero
Site of infection			
Abomasum			
Haemonchus contortus	✓		
Teladorsagia circumcincta	✓		
Ostertagia ostertagi			✓
Trichostrongylus axei	✓		
Small intestine			
Cooperia curticei		✓	
C. oncophora			✓
Nematodirus filicollis	✓		
N. spathiger	✓		
T. colubriformis	✓		
T. vitrinus	✓		
Large intestine			
Chabertia ovina		✓	
Oesophagostomum venulosum		✓	
Trichuris ovis		✓	

(Adapted from Pomroy, 1997)

2.3 Effect of parasitic infection on the host

It is perhaps not surprising that the major effects of gut dwelling parasites relate to feed intake and feed utilisation. The consequence of this is most clearly seen as a reduction in the live weight (LW) gain of the growing animal but it also manifested as a reduction in soft tissue development and a reduction in wool

growth and milk production (Brunsdon *et al.*, 1986; Parkins and Holmes, 1989; Holmes, 1993).

2.3.1 Reduction of feed intake

Reductions in feed intake of between 10 and 30% have been observed by many workers (Leyva *et al.*, 1982; Sykes *et al.*, 1988; Dynes *et al.*, 1991) but the precise reasons for this parasite induced anorexia remain unclear (Symons, 1985). Whether it is simply a response to abdominal pain or some other physiological factor such as a change in gut motility and flow rate of digesta has yet to be determined. Measuring degrees of pain and relating them to voluntary feed intake may be a difficult exercise to undertake. Recent studies investigating parasite induced anorexia have examined the role of central satiety signals including neuropeptides. Dynes *et al.* (1990) indicated that the reduction in feed intake could be overcome by blocking these signals from the hypothalamus. The involvement of neuropeptides in the stimulation and inhibition of appetite in parasitised animals was also investigated by Horbury *et al.* (1995). Their work indicated that increased gene expression of neuropeptide Y (a stimulator of appetite) was associated with anorexia in the early stages of infection of rats with the gut dwelling nematode *Nippostrongylus brasiliensis*. This suggested that periods of energy deficit associated with parasite induced anorexia, could trigger stimuli to increase feed intake but the workers failed to show the opposing stimuli which depressed appetite. Further investigation of the factors involved in intake stimulation are required.

2.3.2 Effects on nutrient digestion and absorption

The impact of parasitic infection on the host has been monitored using pair feeding studies (Barger *et al.*, 1973; Sykes and Coop, 1976; Sykes and Coop, 1977). Feed intake of infected animals is recorded and similar levels are offered to pair-fed non-infected individuals. Studies using this technique indicate that it

is not anorexia alone which is responsible for reduced productivity of infected animals. Pair-fed non-infected animals have been shown to perform better than their infected counterparts. Weight gain in lambs was reduced by 20% and 50% compared to pair fed controls, as a result of *O. circumcincta* (Sykes and Coop, 1977) and *Trichostrongylus colubriformis* (Sykes and Coop, 1976; Kimambo *et al.*, 1988) infections, respectively. This suggests that in addition to inappetence, there is a reduction in the efficiency of feed digestion and absorption.

Initially, it was thought that much of the productive penalty occurring (in addition to that caused by reduced feed intake) was related to malabsorption of nutrients and losses of endogenous protein (Steel, 1978). The physical damage caused by parasite activity, including the formation of lesions, destruction of gastric glands and flattening and stunting of microvilli, was considered to greatly reduce the efficiency of nutrient absorption (Dargie, 1980). Studies indicated that parasitised animals had an increased level of N in digesta, at their terminal ileum (Poppi *et al.*, 1986; Kimambo *et al.*, 1988) but it was unclear as to whether this reflected a reduction in protein digestion and absorption within the small intestine or an increase in the level of endogenous protein in the gut from sloughed epithelial cells, increased plasma leakage and mucous secretion. It was concluded by Poppi *et al.* (1986) that the latter was probably the major contributor since ^{35}S labelled microbial protein infused into the abomasum appeared to be absorbed to the same degree in both infected and non infected animals.

Nitrogen balance studies have shown that the majority of protein in the GI tract is reabsorbed before the terminal ileum (Symons and Steel, 1978; Poppi *et al.*, 1986). The reabsorption of protein from sloughed cells and mucin secretion tends to be greater in abomasal than in small intestinal infections and clearly there will be an energy cost associated with this recycling. This is reflected in the gross efficiency of use of metabolisable energy (ME) for energy deposition in both abomasal and small intestinal infections (Sykes and Coop, 1976; Sykes and Coop,

1977). The partitioning of nutrients towards gut repair will adversely affect the productivity of the animal. Interestingly, it is infection of the small intestine which appears to have the greatest impact on nutrient utilisation while reductions in gross efficiency of use of ME for energy retention, caused by infection of the abomasum, are considered to be far less severe (Sykes *et al.* 1988).

2.3.3 Effects on mineral metabolism

Gastrointestinal parasitism has also been reported to adversely affect bone growth in sheep (Sykes *et al.*, 1977; Sykes *et al.*, 1979), which suggests that the absorption of phosphorus and/or calcium may be diminished in infected animals (Sykes *et al.*, 1975). The uptake and retention of phosphorus is reduced in animals infected with the intestinal *Trichostrongylus* spp. but not by the abomasal *Teladorsagia* spp. (Table 2.1) (Reveron *et al.*, 1974; Wilson and Field, 1983). The effect on calcium uptake is less clear. Increased endogenous losses of Ca, resulting from infection, have been observed (Wilson and Field, 1983), as have reductions in plasma calcium concentrations. Evidence of compensatory absorption of calcium further down the alimentary tract may indicate that intestinal parasitism has a direct effect on calcium absorption (Poppi *et al.*, 1985; Bown *et al.*, 1989).

2.4 Production losses resulting from gastrointestinal parasitism

Young stock

Clearly the reduction in feed intake and the negative effects of parasitic infection on feed utilisation have a significant effect on the productivity of the host. From a very early age young lambs will graze contaminated pasture while suckling the ewe. Lamb growth rate is adversely affected by parasitic infection (Brunsdon, 1966; Coop *et al.*, 1982; Kimambo *et al.*, 1988). Reductions of up to 50% in LW gain and feed conversion efficiency have been estimated in growing lambs ingesting less than 600 nematode larvae kg^{-1} of fresh herbage day^{-1} (Sykes, 1994).

Coop *et al.* (1985) demonstrated that the reduction in LW gain increased with increasing larval challenge. In their study, cross bred lambs trickle infected from three-and-a-half months of age with either 1,500, 3,000, or 5,000 *O. circumcincta* larvae, were found to have LW gains of 115, 104 and 95 g day^{-1}, respectively, compared with a LW gain of 151g day^{-1} for uninfected control lambs. Such reductions result in a failure to reach finished weight within a given time period and may have financial implications to a producer if target LW of stock are not reached when market returns are at their highest. Indeed, Coop *et al.* (1985) suggested that lambs experiencing moderate levels of infection of about 3,000 *O. circumcincta* day^{-1} could take between four and seven weeks longer to reach target slaughter weight of 36 to 38 kg than lambs exposed to lower levels of challenge.

Adult stock

Losses are not only restricted to young stock. Brunsdon *et al.* (1986) reported significant depression of LW gain (2.4 kg) in breeding ewes grazing pasture of larval contamination approximating 4,400 larvae kg^{-1} pasture, compared with ewes grazing considerably less contaminated pasture of approximately 33 larvae kg^{-1}. In addition to reduced LW gain, parasitised sheep have also been reported as experiencing reduced milk yield (Thomas and Ali, 1983; Sykes and Juma, 1984) and decreased wool production (Barger *et al.*, 1973; Barger and Southcott, 1975; Brunsdon *et al.* 1986). Leyva *et al.* (1982), found that food intake of lactating ewes was reduced by 16% as a result of *O. circumcincta* infection. The infection also had an adverse effect on milk production - reduced on average by 270 g day^{-1}, and on wool production. Wool growth and fibre diameter were reduced by 20 and 7%, respectively.

2.5 Immunity to gastrointestinal parasitism

2.5.1 Mechanisms of immunity

Unlike the effective immune response mounted against bacterial and viral infections, young lambs do not appear to be born with a natural innate (non-specific) immunity to parasitic infection of the GI tract (Soulsby, 1981; Smith *et al.*, 1985). Rather, immunity is acquired, over a period of time, with exposure to infective larvae of the many helminths known to inhabit the GI tract (Refer Table 2.1). Immunity to such parasitism is slow to develop, seldom provides complete resistance to infection and is believed to diminish with time if the challenge is withdrawn (Dineen and Wagland, 1966; Wagland and Dineen, 1967). The precise mechanisms of immune facilitation are complex and not fully understood. It has, however, become apparent that host age is important in the development of resistance. Young lambs, below the age of six months, appear to be more susceptible to parasitic infection than older animals (Gibson and Parfitt, 1972; Dobson *et al.*, 1990; Kambara *et al.*, 1993). As the animal ages a number of components of immunity are believed to become active. These are discussed below.

Innate immunity

Innate immunity refers to the various defensive barriers associated with initial infection - gut endothelial wall and mucus, inflammatory responses, gut motility and phagocytosis. These responses are non-specific and act against repeated infection of a wide range of invasive organisms (McFarlane, 1997).

Acquired immunity

Acquired immunity involves the ability of the host to recognise specific invasive organisms (including parasites) and to act selectively to eliminate these. This encompasses both humoral (antibody production) and cellular (specific cell production) immunity (Miller, 1984). In terms of parasite specific antibodies the

major immunoglobulins associated with humoral immunity appear to be IgA, both to *Haemonchus contortus* (Smith, 1977) and *T. colubriformis* infection (Cripps and Rothwell, 1978), and IgG, the activity of which has been detected in both *Haemonchus* and *Teladorsagia* spp. infected adult sheep (Smith, 1977; Smith *et al.*, 1983). The concentration of different antibodies appears to vary within serum, intestinal fluid, mucus and mucosal extract depending on the antibody function (Dobson, 1966a; Dobson, 1966b; Dobson, 1967). IgG appears to be the major antibody present in the bloodstream but it is also produced locally in plasma cells in the lamina propria of the alimentary tract (Wakelin, 1984; Crook, 1990). Consequently, IgG activity appears to be associated with activity at a local level, particularly in gut, mammary gland and bronchi tissue (Nansen, 1985). The presence of IgA in the mucosae of lambs resistant to *H. contortus* infection suggests that this antibody is important at a local level (Duncan *et al.*, 1978).

Many of the mucosal secretions contain immunoglobulins. These secretions are believed to physically impede parasitic larvae, possibly through preventing their burrowing into the gut lining. Evidence of the inhibitory nature of mucosal secretions was demonstrated by Douch *et al.* (1983) and later by Kimambo and MacRae (1988), who reported that larval migration *in vitro* was inhibited on agar gels which contained mucus from sheep resistant to parasitic infection but not by gels containing mucus from susceptible sheep. The precise mediator of this inhibition, for example histamine, dopamine or adrenalin, has yet to be isolated. It is understood that immunoglobulins protect the host from parasite activity through antibody mediated, neutralisation of proteolytic enzymes, used by the larvae (Tizard, 1982). As humoral immunity depends on the production of antibodies in response to antigen stimulation, effectiveness is achieved only after repeated periods of infection.

Cellular immunity

Cellular immunity has also been shown to play an important part in the resistance of sheep to GI parasitism. Smith *et al.* (1984) demonstrated that resistance could be conferred upon a previously susceptible sheep by transferring lymphocytes from the gastric lymph of genetically identical resistant twins. However, the mechanisms by which lymphocytes effect the resistance of animals remains poorly understood, to the extent that worm expulsion may be caused by some other, as yet unknown, cell type (Miller, 1984). It is believed that T (thymus derived) lymphocyte cells are important in helminth infection and may secrete cell regulators which lead to the production of IgG, IgA and IgE from plasma cells and increase the production of eosinophils (Finkelman *et al.*, 1991). Two classes of T cell have been linked to immunity to parasites - T helper (CD4+) and gamma delta (WCI) cells. Gill *et al.* (1993) demonstrated the failure of animals to develop resistance to *H. contortus* infection where CD4+ cells were depleted and Kambara and McFarlane (1996) found that these cells were associated with resistance to *T. colubriformis* infection in young lambs. Kambara and McFarlane (1996) demonstrated an inverse relationship between WCI cell numbers and resistance to *T. colubriformis.*

The acquisition of immunity has also been associated with the presence of large numbers of mucosal mast cells - the density of which reportedly increases significantly at the time of infection (Miller *et al.*, 1981; Rothwell, 1989). It has been hypothesised that the proliferation of mucosal mast cells in response to parasitic challenge is under cytokine control (Finkelman *et al.*, 1991). These cells appear to respond to parasite antigen and release vasoactive substances (histamine, leukotrienes, and prostaglandin E_2) which are believed to inhibit larval migration within the host gut (Douch *et al.*, 1996). Indeed, Stankiewicz *et al.* (1993) demonstrated a strong correlation ($r = 0.92$) between numbers of globule leucocytes (a likely modified mast cell) and larval migration inhibition in mucus following *T. colubriformis* infection in sheep. Interestingly, mucosal mast cell production and subsequent conferred resistance, has been shown to be

abrogated by elevated levels of corticosteroids (Miller and Huntley, 1982; Bell *et al.*, 1982).

Basophils and eosinophils, which arise from bone marrow also appear to be associated with mucosal activity against nematodes (Kyriazakis *et al.*, 1996). van Houtert *et al.* (1995) found that there was a marked peripheral eosinophilia by approximately the eighth week of *T. colubriformis* infection in lambs infected from three months of age.

Summary

In summary, the host animal's defense against GI parasitism involves a complex array of mechanisms. Antibodies may be produced against various parasite antigens and because of the diverse nature of these parasites and the different niches they inhabit within the gut, it seems likely that defence mechanisms will be equally varied and site specific. The many cells involved, including lymphocytes and mucosal mast cells, are unlikely to act alone in conferring resistance - more realistic is the hypothesis that complex inter-relationships exists between the different components, which eventuate in the expulsion of parasites.

2.5.2 Manifestation of host resistance

The consequence of the various immune responses described above were summarised by Miller (1984), as resulting in either an expulsion of the parasite or it's persistence but in an adapted and often impaired form. Absolute resistance could be considered as a complete failure of larvae to establish, but as stated previously, the immune response is seldom this effective (Nansen, 1985). Generally there is a build up of infection, in the young animal, as resistance develops in the first six months of life (Gregg and Dineen, 1978).

Rejection of incoming larvae

With acquired immunity, comes the ability to reject incoming larvae within the first 24-48 hours of infection - described by Miller (1984) as rapid expulsion. The factors controlling rapid expulsion remain unclear but in studies with rats infected with *Trichinella spiralis*, it is believed that both specific systemic immunity and a local, probably non-specific immune response were required for rapid expulsion to take place (Bell *et al.*, 1979; Bell and McGregor, 1980a; Bell and McGregor, 1980b). Rapid expulsion was also demonstrated in *T. colubriformis* infection in lambs (Chiejina and Sewell, 1974) and in *H. contortus* infection, where it appears to be associated with a failure of larvae to migrate through mucosa to their predilection sites in gastric glands (Miller *et al.*, 1983).

Rejection of established worms

Immune responses are also observed against established larvae and adult worms. Michel (1970), hypothesised that the expulsion rate of adult *O. ostertagi* worm burdens in calves was proportional to the number of worms present. This may suggest that a threshold adult worm burden is required to activate the rejection of larval establishment. This theory was supported by both Chiejena and Sewell (1974a) and Jackson *et al.* (1983), who observed a decrease in the level of larval establishment with increased adult worm burden.

Reduced fecundity

Reduced worm fecundity is also reported as a consequence of host resistance in many species of animal (Krupp, 1961; Michel, 1963; Bell *et al.*, 1982). In mice infected with *N. spiralis* it was found that the fecundity of worms could be reduced in susceptible hosts through the transfer of lymph node cells from resistant individuals (Wakelin and Wilson, 1980).

Inhibited development

Immune mediated inhibition of development was defined as "a temporary cessation of development of nematodes at a precise point in early parasitic development" (Michel, 1974). It has been shown to be more prevalent in animals which have received repeated infections than those receiving similar numbers of larvae as a single infection (Donald *et al.*, 1964; Dineen *et al.*, 1965) thus indicating that inhibition may be a host induced response. This was further demonstrated by Dunsmore (1961), who found that cortisone treatment of sheep challenged with *Teladorsagia* spp. substantially reduced the proportion of arrested larvae.

Larval stunting

Resistance has also been reported to stunt growth of larvae, as distinct from inhibited or arrested development. Evidence that this occurs as a consequence of host immunity was provided by Dobson (1982) who found that the effect could be transferred from resistant to susceptible hosts using immune serum.

2.5.3 Factors affecting resistance to gastrointestinal parasitism

Host genotype

There are several reports of genetic variation in the level of and rate of development of acquired immunity, both within and between sheep breeds (Altaif and Dargie, 1978a, 1978b and Reviews by Dargie 1982; Gruner and Cabaret, 1988; Gray, 1991). Scottish Blackface sheep are considered to be relatively resistant to *H. contortus* infection (Altaif and Dargie, 1978a, 1978b; Abbott *et al.*, 1985a, 1985b) while Hampshire Downs are relatively susceptible (Loggins *et al.*, 1965; Preston and Allonby, 1979). A number of the immunological responses observed to both *Trichostrongylus* and *Haemonchus* spp. infection, in sheep, have been shown to be under genetic control (Windon, 1996). It is believed that high responder animals may have greater antigen recognition and enhanced effector responses such as mast cells and circulating eosinophils

(Windon, 1996). Gill *et al.* (1993) were able to induce susceptibility in a genetically resistant lamb by depletion of CD4+ cells by monoclonal antibodies, which they also found was associated with reduced numbers of mucosal mast cells, globule leucocytes and eosinophils.

At present there is considerable interest in the specificity of genetic resistance and whether selection for resistance to one parasite species is likely to improve protection against other species. It has been hypothesised that the mechanisms involved in facilitating immune responses differ between parasite species thus reducing the likelihood of non specific genetic resistance (Windon, 1991). There is clearly a need for more in-depth research in this area.

Host age

Growing lambs, less than six months of age, acquire immunity to GI parasitism more slowly than older sheep (Gibson and Parfitt, 1972; Dineen *et al.*, 1978; Bown *et al.*, 1991; van Houtert *et al.*, 1995). The reason for this remains unclear but it has been attributed to impaired antibody responses, such as antibody production (Duncan *et al.*, 1978), impaired lymphocyte responsiveness to parasite antigen (Riffkin and Dobson, 1979) and changes in the population of globule leucocytes in the intestinal mucosa (Gregg *et al.*, 1978). Many of the components of immunity are proteinacious in nature and it has been suggested that the apparent lack of immonocompetancy observed in young lambs may reflect competition for available nutrients between the requirement for growth and requirements to mount an effective immune response (Coop and Holmes, 1996). Further work is required before this can be substantiated.

Host nutrition

Early studies investigating the relationship between host nutrition and resistance to GI parasitism, although simplistic in their approach, provided evidence of two distinct effects. Firstly, sheep on an enhanced nutrient plane appeared better able to withstand the pathophysiological consequences of infection (Clunies-Ross and Graham, 1932; Clunies-Ross and Gordon, 1933; Lucker and Neumayer, 1947) - in other words, the host was more resilient to the effects of the disease than less well fed counterparts. Secondly, well fed sheep were better able to fight-off infection and reduce parasite burdens than less well fed sheep (Fraser and Robertson, 1933; Taylor, 1934) - improved nutrition apparently enhanced resistance to infection.

More recent studies have shown that it is protein nutrition which appears to be particularly important in the development of both resilience and resistance to infection. In a detailed study by Bown *et al.* (1991), lambs receiving abomasal infusions of protein (50 g day^{-1}) were more able to overcome the reduction in energy and protein deposition resulting from infection of 3,000 *T. colubriformis* larvae day^{-1}, than lambs abomasaly infused with isocaloric amounts of glucose - suggesting that GI parasitism induces a protein rather than an energy deficiency.

Prior to the work of Bown *et al* (1991), other workers had reported that protein supplementation did not appear to influence the establishment of parasitic infection (Bawden, 1969; Dobson and Bawden 1974). In these studies sheep were offered either low protein (60 g CP kg^{-1} DM) or high protein (120 g CP kg^{-1} DM) rations and later infected with *Oesophagostomum columbianum.* Results indicated that initial establishment of infection was unaffected by protein supply. This was confirmed in subsequent studies (Bown *et al.*, 1991; van Houtert *et al.*, 1995), using *T. colubriformis*, where it was concluded that protein supplementation

appeared to have little effect on the development of innate immunity. However, the results from these studies and many others, have suggested that protein nutrition appears to affect the rate of acquisition of an effective immune response in the face of challenge (Duncombe *et al.*, 1981; Abbott *et al.*, 1988; Bown *et al.*, 1991; van Houtert *et al.*, 1995). In a recent study by Coop *et al.* (1995), lambs, approximately seven months of age, were capable of mounting a more effective immune response against a challenge infection of 50,000 *O. circumcincta* larvae, following a period of by-pass protein supplementation, than non-supplemented lambs. Both groups of lambs had received a trickle infection of 2,000 *O. circumcincta* larvae during the eight weeks preceding the challenge, which commenced when the animals were approximately four and a half months of age. The supplemented lambs had been abomasally infused with 45 g of sodium caseinate day^{-1}. Worm burdens resulting from the challenge infection were lower in protein supplemented lambs which correlated with higher concentrations of mucosal mast cells, thus suggesting an enhanced immune response.

It is of interest to note that the resistance conferred on sheep resulting from protein supplementation has been reported to vary between breeds (Abbott *et al.*, 1985a, Abbott *et al.*, 1985b; Wallace *et al.*, 1995; Wallace *et al.*, 1996); with age of the host (Abbott *et al.*, 1988; Abbott and Holmes, 1990; Kambara *et al.*, 1993); and with the type of infection - single challenge vs. trickle (Abbott *et al.*, 1986; Abbott *et al.*, 1988). This was alluded to by Coop and Holmes (1996) in their review of nutrition and parasite interactions, which suggested that there may well be additive effects of these factors on immune responses. Abbott *et al.* (1985a, 1985b) demonstrated that the response to protein supplementation both in terms of resistance and resilience to GI parasitism appeared to be greater in Finn Dorset sheep (a breed considered to be relatively susceptible to *Haemonchosis*) than in Scottish Blackface sheep. Using soyabean meal to enhance the protein supply, three month old lambs of each breed were fed either 170 g CP kg^{-1} DM day^{-1} or

88g CP kg^{-1} DM day^{-1}. The lambs were slaughtered 20 weeks after challenge infection of 125 *H. contortus* larvae kg^{-1} body weight. In the 'resistant' Scottish Blackface group there was no effect of diet on *H. contortus* establishment or on the pathophysiological changes associated with infection. However, in the 'susceptible' Finn Dorset lambs faecal egg counts (FECs) were significantly higher in sheep on the low protein diet and these lambs exhibited the severest pathophysiological effects of the disease (Abbott *et al.*, 1985b).

The results of these studies suggest that where an animal is genetically susceptible to infection then an additive response can be achieved through dietary supplementation and in contrast where the animal is resistant it appears that nutrition is unlikely to further enhance resistance. A similar effect appears to occur with age related resistance. As outlined previously young lambs below 6 months of age are unable to mount as effective an immune response to GI parasites as older animals (Nansen, 1985). Kambara *et al.* (1993) demonstrated that young lambs (2 - 6 months of age) offered protein supplemented diets (200 g CP kg^{-1} DM) showed greater resistance to *T. colubriformis* infection than similar aged lambs offered basal diets (111 g CP kg^{-1} DM). However, protein supplementation did not have this effect in older lambs (7 - 12 months of age). Again this suggests that protein supplementation is effective in particularly susceptible individuals.

It is evident therefore, that resistance to GI parasitism is influenced by a number of factors, including host genotype, age and nutrient intake. The precise relationship between the acquisition and maintenance of an immune state and these host factors remains to be elucidated.

2.6 The periparturient breakdown of resistance to gastrointestinal parasitism

Pregnancy and lactation have a marked effect on the mammalian host's immunological responsiveness to a number of bacteria, viruses, protozoa and helminths (Lloyd, 1982). In sheep, the breakdown of resistance to GI parasitism is manifested as a rise in the faecal egg output - termed the periparturient rise (PPR), and has been reported to occur both prior to (Brunsdon, 1964; O'Sullivan and Donald, 1970, 1973; Gibbs and Barger, 1986) and after parturition (Brunsdon, 1970; Brunsdon and Vlassoff, 1971; Reid and Armour, 1975). The mechanisms responsible for the increase in FECs and worms burdens remain unclear but it is hypothesised that they may relate to a resumption in the development of previously inhibited larvae, a relaxation in the ability of the host to reject incoming larvae, an increase in the fecundity of female worms or a combination of all three (O'Sullivan and Donald, 1970; Lloyd, 1982; Gibbs and Barger, 1986).

2.6.1 Possible factors leading to periparturient breakdown

Lack of antigenic stimulation

Early workers associated the PPR with a waning of resistance during the winter months due to an absence of antigenic stimulation (Soulsby, 1957). This now seems unlikely as larvae, particularly *Teladorsagia* spp., have been reported to remain present on pasture, in significant numbers, throughout the winter and early spring months (Connan, 1968; Reid and Murray, 1973; McAnulty and Familton, 1993). Pasture larval concentrations of approximately 3,000 larvae kg^{-1} fresh pasture have been recorded on the Lincoln University Research Farm in mid winter (McAnulty, Pers. Comm. Appendix 2.1). Furthermore, the PPR has been reported to occur in tropical regions where larvae are available on pasture throughout the year (Carmichael, 1993; Romjali *et al.*, 1997).

Seasonality of larval activity

The PPR was also thought to relate to seasonal changes in larval activity. Arrested development of larvae is commonly observed in domestic animals (Reid and Murray, 1973) and one hypothesis is that it is triggered by environmental stimuli affecting free-living larval stages in the autumn (Connan, 1968). The PPR was considered by many, to reflect a re-emergence of these inhibited larvae (Field *et al.*, 1960; Connan, 1968; Arundel and Ford, 1969). It was argued however, that this could not have been the sole factor involved in the PPR since studies which eliminated inhibited stages, through anthelmintic treatment prior to lambing, still observed a PPR (O'Sullivan and Donald, 1970; Donald *et al.*, 1982).

Endocrinological suppression of resistance

The role of the lactogenic hormone, prolactin, has been the subject of much research attention - due mainly to the association between increased susceptibility to nematode infection with increased levels of prolactin secretion (Lloyd, 1982). There is certainly evidence to indicate that an association exists - shown both in natural parturition, comparing lactating and non-lactating ewes (O'Sullivan and Donald, 1970; Brunsdon and Vlassoff, 1971) and also in ewes where lactation was induced artificially (Blitz and Gibbs, 1972; Connan, 1973). However, despite the strong synchrony between prolactin secretion and decreased immunosuppression, there is little evidence to suggest prolactin is involved in initiation of the PPR. In a study which looked specifically at the role of prolactin levels and their relationship with the PPR, Coop *et al.* (1990) found that the rise in egg output and therefore the presumed relaxation of resistance occurred prior to the peak of prolactin secretion. These workers also induced lactation in non-pregnant sheep using oestradiol-17ϑ, progesterone and dexamethasone and found that despite elevating plasma prolactin levels significantly above those of non-pregnant control ewes, the non-pregnant lactating ewes remained resistant to nematode infection. The authors thus

questioned the involvement of prolactin as the initiator of the PPR (Coop *et al.*, 1990).

In a further study investigating the role of prolactin in the pathophysiology of the PPR, Jeffcoate *et al.* (1990) significantly suppressed prolactin secretion in lactating ewes using bromocryptine (an antagonist of prolactin) but found that this had no effect on the rise in FECs or on plasma pepsinogen levels. Interestingly though, the suppression of prolactin had no effect on lactogenesis, highlighting the fact that several other endocrinological components, including progesterone (Szekeres *et al.*, 1981) and corticosteroids (Connan, 1973) are involved at this time and may well contribute to immunosuppression. This is clearly an area which deserves further research attention.

Ewe nutrition

The involvement of nutrition in the PPR has been the subject of research speculation for more than 40 years (Morgan *et al.*, 1951; Wilson *et al.*, 1953). These workers postulated that malnutrition, a common occurrence in hill sheep during late winter, was of prime importance in the aetiology of the PPR. The profound loss of body weight experienced by these sheep and the elevated energy demands associated with the peak of lactation were considered to be the primary cause of the breakdown in resistance (Paver *et al.* 1955). This was later questioned however, by Soulsby (1957) who commented on the occurrence of the PPR in lowland ewes, in which malnutrition was seldom observed.

Since these early studies the role of nutrition in the periparturient breakdown has received relatively little research attention. In a study undertaken by Connan (1971), the role of the gross level of nutrition in the post parturient rise was examined. Ewes were either offered diets *ad libitum* (High Plane) or in restricted amounts (Low Plane) and trickle infected with approximately 8,000 *O. circumcincta* larvae week^{-1}, from five weeks before parturition and for the first

five weeks of lactation. A non-pregnant control group was similarly infected throughout the trial period. Within the low plane group, half of the ewes were injected daily with 2 mg thyroxine sodium, which was intended to exacerbate body weight loss by increasing the degree of protein depletion. All ewes were slaughtered six weeks after lambing. Faecal egg counts were lower in the non-pregnant and high plane sheep than in the low plane ewes during lactation but differences were not reported as being significant. No indication of total daily faecal output was given and differences in FECs may have reflected variations in the dilution/concentration of nematode eggs in the faeces. Worm burdens were significantly higher in low plane sheep ($P < 0.05$) than in high plane but the difference in worm burdens between low plane sheep resulting from thyroxine treatment was not significant, despite large differences in body weight loss between these two groups. The author (Connan, 1971) suggested that susceptibility to parasitism by *Teladorsagia* spp. was increased when the plane of nutrition was inadequate. It was, however, apparent that even in ewes on the high plane of nutrition, worm burdens were significantly greater than in the highly resistant unbred sheep. Nitrogen balance studies were not undertaken in the Connan (1971) work, although he hypothesised that differences in protein depletion were highly probable between high and low plane sheep. Despite this, immune suppression had occurred in all pregnant sheep. It was concluded that nutrition was unlikely to be the primary cause of immune suppression associated with lactation.

Many of the advances in elucidating the interaction between host nutrition and resistance to GI parasitism in domestic animals have concentrated on the young growing animal - where both nutrition and parasitism have a very direct effect on productivity and farm income. The consequence of parasitism in ewes around parturition may appear less direct to producers and short-term depression in wool and milk production have, in the past, been effectively remedied by anthelmintic treatment. Added to this is the relative expense of parasite trials involving pregnant sheep and so the paucity of research studies in

this area is perhaps to be expected. The current situation with regard to the increasing incidence of anthelmintic drench resistance and the need to research alternative approaches to parasite control measures clearly justifies greater investigation of the role of nutrition in the PPR, particularly in light of the contribution of the ewe to pasture larval contamination.

2.7 Summary

Grazing sheep are exposed to GI parasites from a young age. As a consequence of this, infected individuals experience a reduction in LW gain and depressed milk and wool production. The young animal appears to have little innate immunity to GI parasitism but as it matures, resistance, in the face of larval challenge, is acquired. There is evidence that the degree and rate of acquisition of resistance is influenced by genetic factors and by host nutrition. The physiology of the immune response remains unclear but resistance to infection is associated with a number of mechanisms including the expulsion of incoming larvae and adult worms; a reduction in the fecundity of female worms; and an inhibition in larval development.

The periparturient period is associated with a temporary relaxation of many of these immune responses. In sheep, this is manifested as a rise in nematode egg output in the faeces of ewes. The larvae which result from this act as a source of infection to young lambs. The precise cause of the periparturient breakdown is unknown but it has been ascribed to many factors including, lack of antigenic stimulation and hormonal immunosuppression. At present there is no evidence to conclusively account for the periparturient breakdown

This thesis describes a series of experiments designed to elucidate the role of nutrition in the susceptibility of periparturient ewes to GI parasitism.

Chapter 3

Body Condition and Current Nutritional Plane

3.1 Introduction

In adult sheep, the breakdown in resistance to GI parasitism is reported to occur at varying times relative to parturition. Gibbs and Barger (1986) observed a rise in FECs in the week prior to parturition which steadily declined in the first month of lactation, while Brunsdon (1970) reported the peak of the rise to occur eight weeks after lambing. The reason for this temporal variation in the relaxation of resistance is unclear. Both Jansen (1968) and Brunsdon (1970) hypothesised that the rise in egg counts at different times reflected changes in the prevalence of different parasite species. In field studies undertaken by Familton *et al.* (1986), where worm populations consisted almost entirely of *Trichostrongylus* and *Teladorsagia* spp., a rise in egg counts was observed in mid pregnancy in one year and during lactation in a subsequent years. These findings question the importance of species specificity in the timing of the periparturient rise.

The nutritional status of the ewe may offer an alternative explanation for the variation in timing of the breakdown. Early work by Clunies-Ross and Gordon (1933) demonstrated that the immune status of mature sheep was compromised by a period of very poor nutrition and a weight loss of between 4.5 and 7.0 kg. This study, however, involved very few animals and the dietary treatments were extreme, *viz.* wheat straw with a crude protein content of 31 g kg^{-1} DM. Connan (1971) reported that worm burdens were significantly lower in high plane compared with low plane ewes but both groups experienced a breakdown in resistance to *Teladorsagia* spp. infection. Protein depletion during lactation, he concluded, was not the primary cause of immune suppression.

The present study was undertaken to determine the effect of changes in LW and body condition of ewes in late pregnancy, achieved by differential energy supply, on the timing and magnitude of the periparturient breakdown of resistance in ewes.

3.2 Materials and methods

Experimental design

Forty-eight mixed-age female Coopworth sheep (Border Leicester X NZ Romney) of mean LW 55 ± 5.5 kg were allocated hierarchically on the basis of LW to two groups 10 weeks prior to parturition. One group was fed to gain (HE) and the second to lose (LE) maternal body weight from nine weeks prior to parturition, the objective being to achieve a 10 kg difference in LW at parturition. The sheep were trickle infected with 4,000 *Teladorsagia circumcincta* infective larvae day^{-1}, from five weeks prior to parturition. In eight animals from each treatment, infection ceased at parturition (group S0) and the sheep were slaughtered. For the remaining animals in each treatment, infection ceased either ten days (group S6; n = 8) or 31 days (group S9; n = 8) after parturition, and the animals treated with anthelmintic. Eleven days after anthelmintic treatment, sheep in these latter 2 groups were given 25,000 *T. circumcincta* larvae as a challenge infection and slaughtered 21 days later.

Feeding and management

From nine to seven weeks prior to parturition the HE and LE sheep were rotationally grazed on separate 0.4 ha plots of ryegrass (*L.pererene*)/white clover (*T.repens*) pasture, at a stocking rate 14 ewes ha^{-1} and supplemented with 500g $head^{-1}$ day^{-1} lucerne hay (refer to Table 3.1 for feed analysis). Crude protein was defined as CP(g kg^{-1}) = g N kg^{-1} x 6.25, where N = nitrogen determined by Kjeldahl extraction. Metabolisable energy content of the forages was estimated using the equation of Barber *et al.* (1984) *viz.*

$$ME\ (MJ\ kg^{-1}\ DM) = 0.0157[DOMD^{1}]\ r^2 = 0.83,$$

[1] DOMD - Digestible organic matter, g kg^{-1} DM

where [DOMD] is as g kg^{-1} dry matter. Low plane and HE groups were further supplemented with 200 or 400g $head^{-1}$ day^{-1} pelleted barley-based concentrate ration, respectively (Table 3.1). Metabolisable energy content of the concentrate was estimated using the equation recommended by Alderman (1985) *viz.*

$$11.78 + 0.0654\ CP\% + 0.0665\ EE^{1}\%^{2} - (0.0414\ EE\% \times CF^{2}\%) - 0.118\ ASH^{3}\%$$

[1] EE – Ether extract (oil) content, g kg^{-1} DM

[2] CF – Crude fibre

[3] ASH – Inorganic constituents of food

Herbage mass declined from 800 to 400 kg DM ha^{-1} during the two week period.

Table 3.1 Composition and analysis of pellets and hay offered to sheep during Trial 1 (g kg^{-1} DM)

	Feed		
	Pellet[1]	Lucerne hay	Meadow hay
Composition			
Barley grain	690		
Malt culms	270		
$CaCO_2$	18		
NaCl	10		
$NaHCO_2$	10		
Analysis			
Dry matter	900	830	850
DOM[2]	818	585	553
Crude protein	124	239	168
Metabolisable energy (MJ kg^{-1})	11.5[3]	9.0[4]	8.0[4]

[1] Propel Feeds Ltd, Christchurch, NZ.
[2] DOM – Digestible organic matter
[3] Estimated per Alderman (1985)
[4] Estimated per Barber *et al.* (1984)

Seven weeks before parturition the sheep were drenched and housed in individual pens on slatted flooring and offered meadow hay (Table 3.1) and increasing amounts of the concentrate. Between weeks 15 and 21 of pregnancy HE sheep were offered an estimated 11.9 MJ ME increasing in regular increments to an estimated 24.0 MJ ME $head^{-1}$ day^{-1} and LE sheep an estimated 7.4 MJ ME increasing in regular weekly increments to an estimated 13.6 MJ ME $head^{-1}$ day^{-1}. The ration consisted of approximately 85% concentrate and 15% hay on a dry matter basis. After parturition all sheep were offered 2.5kg concentrate and 400g chaffed meadow hay, irrespective of group. This provided approximately 28 MJ ME $head^{-1}$ day^{-1} throughout lactation. Feed refusals were recorded from three weeks prior to

parturition for estimation of intake. Total daily faecal output was estimated as outlined in Appendix 3.1.

General methodology

Ewe LW was recorded 10 weeks prior to parturition and then at weekly intervals from eight weeks prior to parturition until the end of the trial. Body condition score (CS) was recorded ten weeks from parturition and then weekly from housing until one week after lambing. Condition score was assessed using the technique described by Russel *et al.* (1969) and was undertaken by the same operator on each occasion. Ewe litter weight was determined by summing the birth weight of lambs recorded as soon as practical after lambing.

Blood analysis

Blood was collected one hour before feeding (Monday and Thursday) at approximately 0900h by jugular venepuncture into 10 ml evacuated glass tubes containing 125 USP sodium heparin (Becton Dickinson, VACUTAINER Systems, Rutherford, New Jersey, USA). Plasma was separated immediately by centrifugation and an aliquot of 2 ml taken for measurement of plasma β-hydroxybutyrate (BHB) concentration (Koch and Feldbruegge, 1987). The remainder was stored at -20°C until plasma pepsinogen analysis was undertaken.

Plasma β-hydroxybutyrate

Plasma β-hydroxybutyrate concentration was determined twice weekly to monitor energy status (Koch and Feldbruegge, 1987). Where BHB concentration exceeded 0.8 and 1.5mmol l^{-1} for the HE and LE animals, respectively, concentrate ration allowance was increased by 200g day^{-1}.

Parasitology

Infective *T. circumcincta* larvae (Kumeroa strain) were originally obtained from AgResearch, Wallaceville Animal Research Centre, Upper Hutt, New Zealand and had been passaged through naive lambs several times. The level of daily trickle

infection was chosen based on the pasture larval data of Vlassoff (1982) which demonstrated that larval concentration on pasture could range from a few hundred to in excess of 30,000 larvae kg^{-1} fresh herbage. Assuming a DM intake of 1.2 kg ewe^{-1} day^{-1}, these levels of larval contamination would produce larval intakes ranging from almost zero to in excess of 200,000 larvae day^{-1}. The level of 4,000 larvae day^{-1} was estimated to be at the lower end of the scale of potential larval intakes for grazing ewes in late winter and early spring under New Zealand conditions (Vlassoff, 1982), but had provided typical pathogenicity of parasitic infection in the studies of Leyva *et al.* (1982) and McAnulty (1990).

Infective larvae were cultured from nematode eggs obtained from the faeces of lambs monospecifically infected with *T. circumcincta.* Larvae were maintained in an aqueous suspension of known larval concentration. The required dose of larvae was pipetted onto moist filter paper and rolled to form a bullet which was administered orally using a bolus applicator gun. Daily doses were combined and administered three times weekly at the same time of day on each occasion, namely, after blood sampling and prior to feeding.

The ewes were faecal sampled two weeks after housing to ensure they were parasite free following the housing drench. Faecal samples were collected twice weekly (Monday and Thursday morning) from five weeks prior to parturition until the sample day before the animal was slaughtered. Sheep in the S6 and S9 challenge infection groups were also faecal sampled 10, 14, 17, 19 and 21 days after receiving the challenge infection. Faecal nematode egg counting was undertaken using a modification of the McMaster method (M.A.F.F., 1979) and results expressed as eggs per gram (epg) of fresh faeces. The dry matter of faeces was determined in the four weeks prior to parturition to enable estimation of total daily faecal egg output.

All ewes were fasted 24 hours prior to slaughter. Slaughter was achieved by stunning with a captive bolt followed by exsanguination by severance of the carotid artery and jugular veins. The abdomen was opened along the midline and the abomasum ligated at the reticulo/abomasal and at the pyloric/duodenal junctions

before removal. Worm burdens were determined in abomasal washings and abomasal digests using the methods of Robertson and Elliot (1966) and Herlich (1956), respectively. Abomasal wash and digest samples were made up to approximately 2000 ml and thoroughly mixed before 4 x 50 ml aliquots were taken, giving a total of 200 ml. To this, 20 ml of formalin was added to give a 10% solution. Counts were made by pipetting a 20 ml aliquot of sample onto a large petri dish with an etched grid base. Contents were examined using a compound binocular microscope and worms differentiated and counted as male and female L4 larvae and L5 adult worms. Total worm burden of each animal was determined by multiplying the total number of worms in each 20 ml aliquot by 110.

Plasma pepsinogen

Plasma pepsinogen concentration was determined to monitor the level of abomasal damage resulting from larval activity in gastric glands. The method of Edwards *et al.* (1960) was adopted but with 0.9% bovine serum albumin (Fraction V, Sigma, St. Louis, MO. USA) in glycine-HCL buffer (0.1 M, pH 2.5) as the substrate (Berghen, 1987). The results were expressed as international units per l of plasma (1 unit = 1 micromole of tyrosine produced per minute). Pepsinogen concentrations were monitored from three days after the start of the trickle infection until parturition.

Statistical analysis

Repeated measures analysis of variance was undertaken on LWT, CS, plasma pepsinogen concentration and faecal egg concentration using the general linear model procedure on the SYSTAT package (SYSTAT, 1990). Additional analysis was carried out where the interaction term with time showed statistical significance. Faecal egg counts and worm burdens were log transformed (Log (count + 1)) before analysis. Feed refusals, feed intake levels, faecal dry matter, litter weight, and worm burdens were analysed using ANOVA on Minitab.

3.3 Results

Of the twenty four sheep allocated to group HE, nine failed to adapt to the diet and were removed from the trial. In the remaining sheep, feed refusals averaged 1.2 MJ ME day^{-1}, in both the HE and LE groups, in the three weeks prior to parturition. Additional feed offered to individual sheep in the LE group in response to elevated BHB concentration did not significantly alter the planned mean daily ration allocation of the group. Refer to Table 3.2 for feed intake data.

Live weight and body condition score

The LW and CS of animals during the trial are shown in Figures 3.1 and 3.2, respectively.

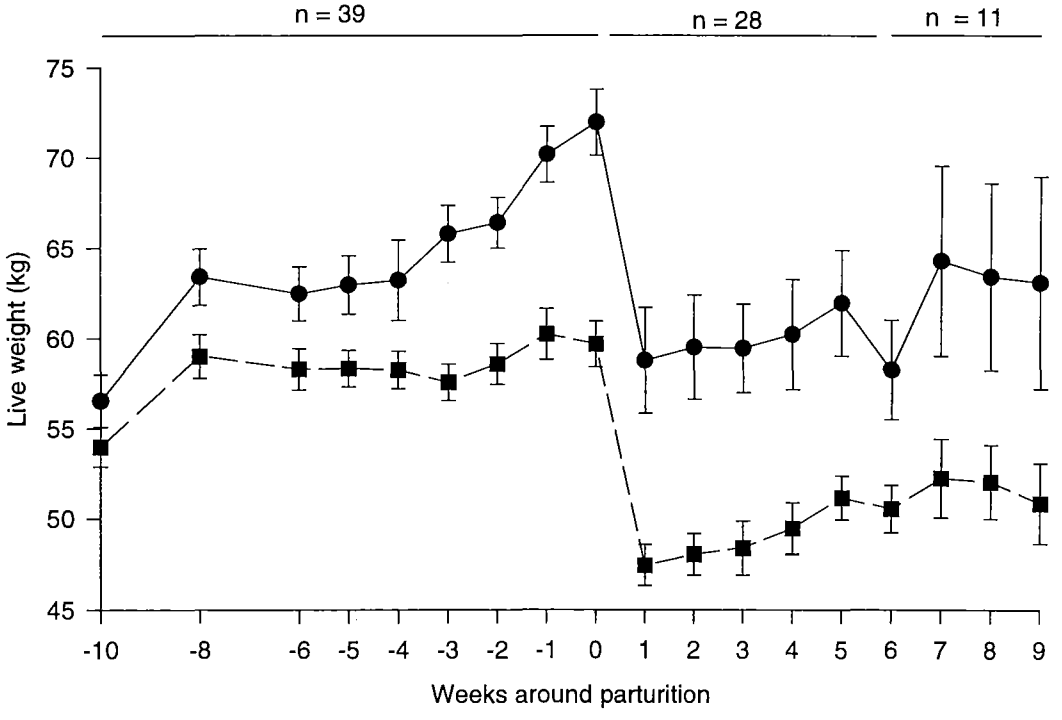

Figure 3.1 Mean live weight of HE (●) and LE (■) sheep around parturition in Trial 1

Table 3.2 Mean daily dry matter (DM) and metabolisable energy (ME) intake of High (HE) and Low (LE) plane groups prior to partution in Trial 1

	HE				LE			
Weeks prior to parturition	DM offered (g)	ME offered (MJ)	DM intake (g)	ME intake (MJ)	DM offered (g)	ME offered (MJ)	DM intake (g)	ME intake (MJ)
6	1290	11.9			850	7.4		
5	1220	13.0			760	8.0		
4	1160	12.8			760	8.2		
3	1160	12.8	1090 ± 42.0	12.1 ± 0.42	760	8.2	640 ± 36.9	7.0 ± 0.37
2	1800	20.3	1660 ± 59.6	18.9 ± 0.60	1060	11.6	940 ± 36.5	10.4 ± 0.36
1	2130	24.0	1990 ± 53.8	22.6 ± 0.54	1240	13.6	1100 ± 32.9	12.4 ± 0.33
0	2560	28.2	2390 ± 65.2	26.5 ± 0.65	2560	28.2	2380 ± 42.7	26.4 ± 0.43

Sheep in HE group gained 15.0 ± 1.71 kg in the ten weeks prior to parturition, while LE group sheep gained only 6.0 ± 1.24 kg in the same period. There was a time by treatment interaction in LW ($P<0.01$) due to this greater weight gain in HE than in LE sheep particularly from 3 weeks prior to parturition. Live weight of HE and LE sheep was significantly different after lambing, *viz.* 58.8 ± 2.93 kg for HE sheep and 47.4 ± 1.12 kg for LE sheep ($P<0.001$) and this was maintained during lactation when LW increased by 3.5 ± 3.35 and 2.8 ± 1.46 kg in HE and LE sheep, respectively.

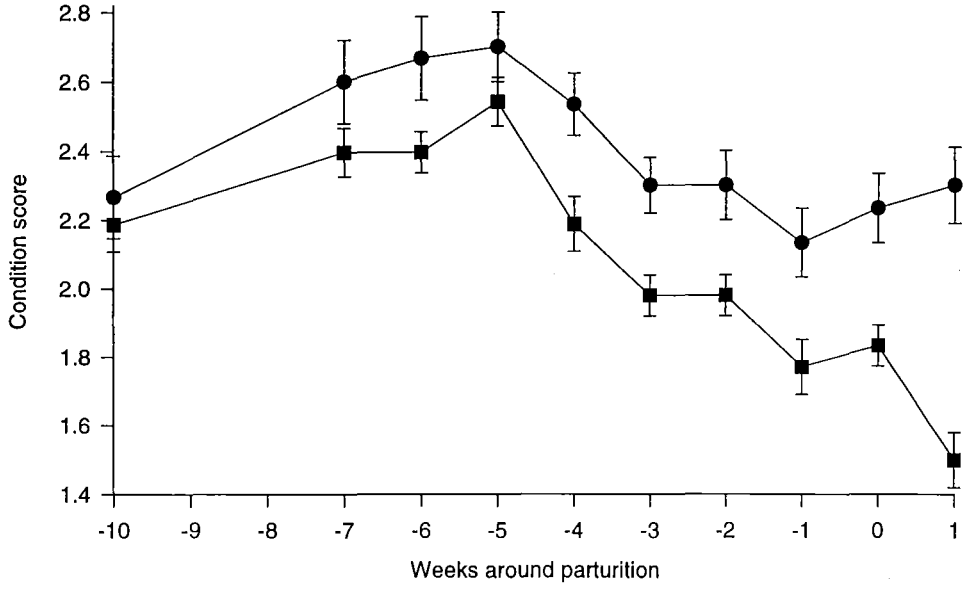

Figure 3.2 Mean condition score of HE (●) and LE (■) sheep prior to parturition in Trial 1

Condition score in both groups increased from the start of the nutritional treatments but then declined from five weeks prior to parturition. Condition score at parturition was significantly different between nutritional treatment groups *viz.* 2.2 ± 0.10 for HE sheep and 1.8 ± 0.06 for LE sheep ($P<0.001$). The overall change in CS of the groups was also significant ($P<0.01$).

Litter weight of lambs born to HE group sheep were significantly higher than those born to LE group sheep *viz.* 9.4 ± 0.40 kg in HE sheep and 7.9 ± 0.26 kg in

LE sheep (P<0.01). Mean litter size was 2.2 and 2.1 lambs for groups HE and LE, respectively.

Parasitology

Geometric mean FECs resulting from trickle infection (Figure 3.3) showed only a significant time effect (P<0.05). However, FECs in LE sheep, were significantly higher than in HE sheep in the week before parturition (P<0.05).

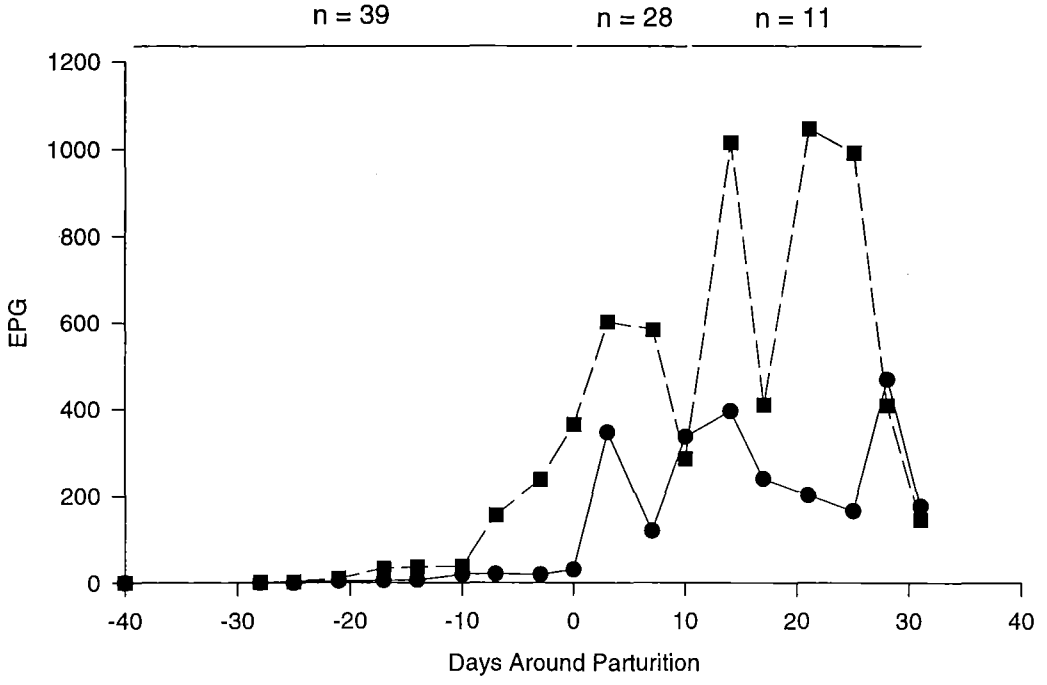

Figure 3.3 Geometric mean (log_{10}(count+1)) faecal egg counts (EPG) of HE (●) and LE (■) sheep resulting from trickle infection in Trial 1

There was no difference in mean faecal dry matter content between HE and LE sheep in the four weeks prior to parturition. Estimated daily faecal output and daily faecal egg output are shown in Table 3.3.

Table 3.3 Estimated mean daily faecal egg output of High (HE) and Low (LE) plane sheep in final four weeks of gestation based on dry matter intake in Trial 1

Weeks prior to parturition	HE			LE		
	Estimated[1] faecal output day^{-1}	Mean EPG[2]	Estimated faecal egg output day^{-1}	Estimated[1] faecal output day^{-1}	Mean EPG[2]	Estimated faecal egg output day^{-1}
3	861	6	5166	507	33	16731
2	1082	19	20558	693	38	26334
1	1470	19	27930	762	237	180594
0	1963	346	679198	1703	601	1023639

[1]Appendix 3.1
[2]EPG - Nematode eggs g^{-1} fresh faeces

Faecal egg counts resulting from the single challenge infections given at either 3 or 6 weeks after lambing are shown in Figures 3.4a and 3.4b, respectively. In both cases egg counts increased with time ($P<0.01$), however, neither nutritional treatment nor time of infection (S6 vs S9) had a significant effect on the geometric mean faecal egg count.

Geometric mean worm burdens of sheep slaughtered at parturition following trickle infection and of sheep slaughtered six and nine weeks post partum following single challenge infections are shown in Table 3.4. None of the differences were statistically significant, there was however a trend toward higher worm burdens in LE sheep ($P=0.08$).

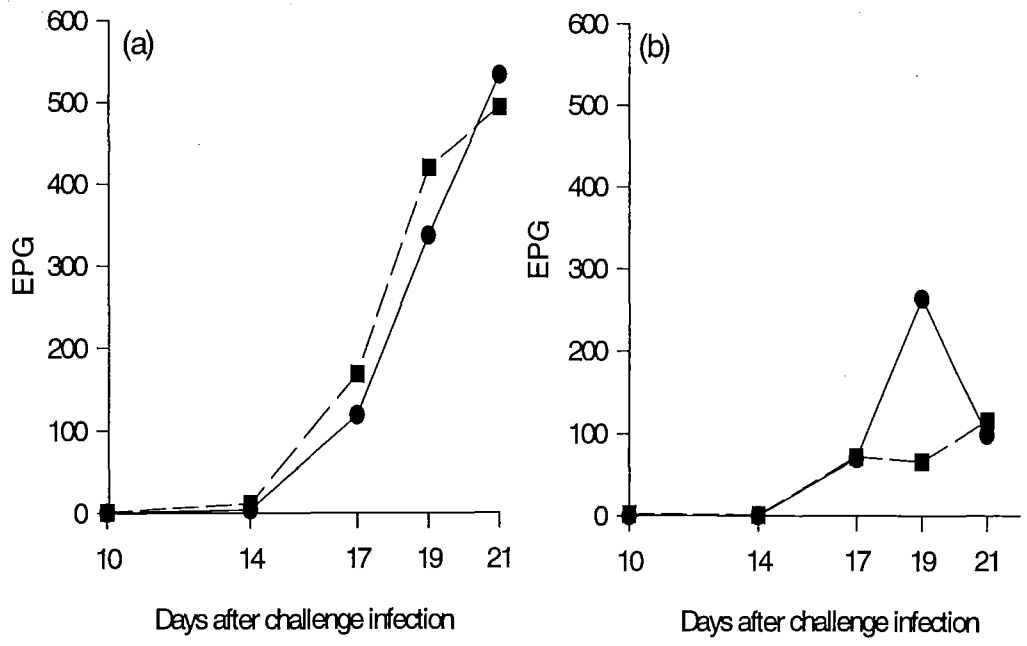

Figure 3.4 Geometric mean (Log_{10}(count +1)) faecal egg count of HE (●) and LE (■) sheep challenge infected 3 weeks (a) and 6 weeks (b) after parturition in Trial 1

Plasma pepsinogen concentration increased significantly with time during the course of the trickle infection in both groups ($P<0.01$) but there was no time by treatment effect (Figure 3.5). Pepsinogen concentration was consistently higher in HE than LE animals but this was significant on only one occasion *viz.* four weeks prior to parturition ($P<0.01$).

Table 3.4 Geometric mean (Log_{10} (count+1)) worm burdens (range) of high (HE) and low (LE) plane sheep slaughtered at parturition and at six (+ 6) and nine (+ 9) weeks post partum in Trial 1

Group	n	Partum (range)	n	Partum + 6 (range)	n	Partum + 9 (range)
HE	5	5800 (1120-25410)	6	3490 (1640-14120)	4	410 (0-11490)
LE	8	18900 (6460-47080)	8	5600 (110-11930)	8	4830 (120-14360)

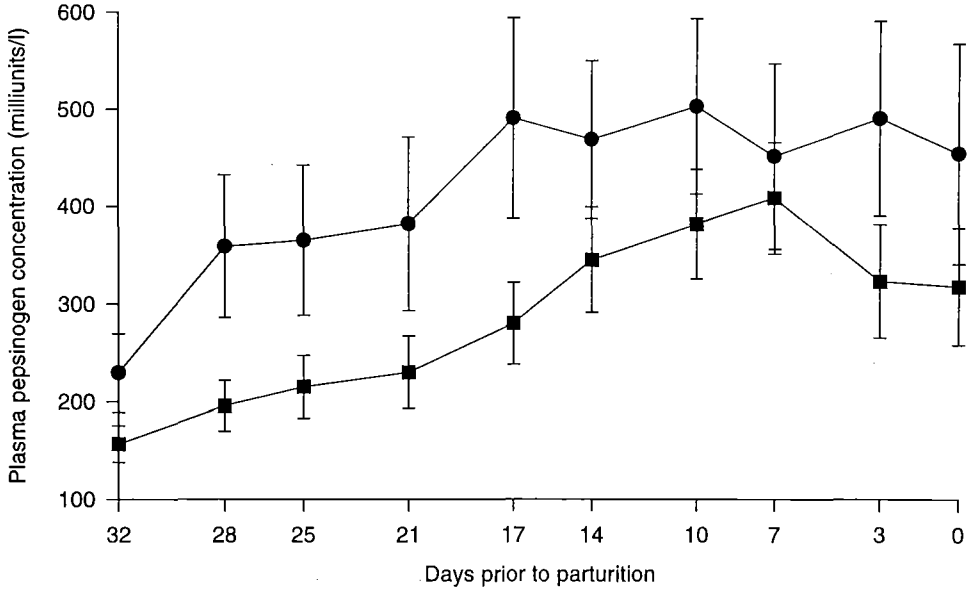

Figure 3.5 Mean plasma pepsinogen concentrations of HE (●) and LE (■) sheep prior to parturition in Trial 1

3.4 Discussion

The results presented clearly indicate that large differences in ewe LW and CS resulting from differing energy provision had little effect on the timing or the magnitude of the periparturient breakdown in resistance to GI parasitism.

The trial design, whereby sheep, in relatively low body condition (2.5), ten weeks prior to parturition, and on a low current plane of nutrition, could be considered extreme both in terms of the nutritional status and treatments imposed. Those in the LE group had initially poor body condition which declined because of the low current plane of nutrition. Indeed Russel (1984) recommended that ewes of CS below 2.5, eight weeks before lambing should be removed from the main flock and receive supplementary feeding, such is the extreme nutritional pressure they are likely to face around parturition. In the present study the differential feeding regime, from ten weeks prior to parturition, would have alleviated this nutritional 'stress' in the HE group to some extent. The LE group however clearly experienced a considerable loss of maternal body weight (Figure 3.1) particularly in the last eight weeks of gestation - average LW of LE sheep in this period decreased by 11.6 ± 1.24 kg compared with a mean LW loss in the same period of only 4.7 ± 2.37 kg for HE sheep ($P<0.01$). This trend was reflected in the 1.5 kg difference in litter weight between LE and HE ewes ($P<0.01$). The increase in LW recorded between week -10 and week -8 was probably a reflection of the low gut fill status of the animals on the first occasion (Week -10). At all other times gut fill variation was minimised by LW being recorded at the same time relative to feeding on each occasion.

Despite the considerable difference in LW between treatment groups and the severity of under nutrition experienced by the LE group, FECs were significantly different only in the week preceding parturition. One could argue that this difference may simply have reflected variation in faecal output

resulting from different feed intake levels. To this end, the estimation of total daily faecal output (Table 3.3) tended to suggest that FECs were greater in LE sheep regardless of faecal output. For example, in the week immediately prior to parturition, faecal output of HE sheep was estimated to be approximately double that of LE sheep, whereas there was a six-fold difference in FECs between LE than in HE sheep *viz.* an estimated daily egg output in excess of 180,000 for LE sheep compared with just under 28,000 in HE sheep. Worm burden data at parturition (Table 3.4) - although tending to suggest greater resistance in the well fed sheep - failed to show any significant differences between treatment groups, clearly questioning the role of energy supply and/or CS in the periparturient breakdown.

The post partum challenge infection was included to determine the role of ewe body condition on the re-establishment of resistance post partum. Results tend to suggest that establishment rates and/or survival of adult nematodes were highest in LE sheep at six weeks post partum (Table 3.4) and that there may have been a reduction in establishment /worm survival with increased body condition and time after parturition. Leyva *et al.* (1982) provided evidence that the magnitude of parasitic infection as measured by faecal egg output decreased as lactation progressed *viz.* averaging 467, 300 and 222 epg for weeks 4, 5 and six of lactation, respectively. One could speculate that this might reflect a resumption of resistance to infection as the elevated nutrient demands of late pregnancy and early lactation begin to lessen - *viz.* ME requirements in the first month of lactation of 30.3 MJ day^{-1} for a 60 kg ewe losing 50g body weight day^{-1}, decreasing to 13.8 MJ by the third month of lactation (AFRC, 1993).

Establishment of larvae from single challenge infection at either 3 or 6 weeks post partum in the present work failed to show statistically significant differences between groups. However establishment rates in HE ewes decreased from 17% in S6 ewes to just 2% in S9 ewes. Although not significant

this trend may indicate a resumption of the immune response which prevents establishment of incoming larvae - a component believed to compromised during the periparturient period (O'Sullivan and Donald, 1970). In contrast the establishment rates observed in LE ewes decreased to a much lesser extent between the two slaughter time periods *viz.* from 28% in S6 to 24% in S9 ewes, possibly indicating that low body nutrient status delayed the return of this immune response. The reduction in worm burdens between the challenge infections 3 and 6 weeks post partum, *viz.* from 3,490 to 410 in HE sheep, and from 5,600 to 4,830 in LE sheep tends to confirm the observations of other workers that normal immune response to parasitism is regained within 12 weeks of parturition or at the end of lactation, whichever occurs first (Michel, 1976; McAnulty, 1990). Interestingly in the work of McAnulty (1990), the highest worm burdens of ewes at pasture were observed 11 weeks after lambing, a time normally associated with re-established resistance. McAnulty (1990) speculated that this had possibly occurred due to the large reductions in body weight experienced by these sheep during lactation, or alternatively that the greater susceptibility reflected a very high pasture larval level. Unfortunately neither were quantified. The interaction between body weight change and larval challenge will be investigated in Chapter 4.

One could speculate that the magnitude of the periparturient breakdown is influenced by the antigenic stimulation encountered by the animals several months prior to lambing. The precise larval challenge experienced by the ewes before they were housed is difficult to quantify, however, Appendix 3.2 gives an estimate of the likely larval intake of the sheep grazing similar pasture, during late summer and autumn.

Plasma pepsinogen results were rather equivocal in that HE sheep recorded consistently, although not significantly, higher concentrations than LE sheep. Plasma pepsinogen concentrations are used as an indicator of abomasal damage resulting from emergence of developing larvae from abomasal glands (Ford,

1976). The difficulty in drawing comparisons between these findings and pepsinogen levels reported by other workers is the tendency toward marked individual variation often observed between trials. Leyva *et al.* (1982) acknowledged this but suggested that the elevation of plasma pepsinogen concentration in their ewes, prior to parturition, to a level of 800 μm l^{-1}, in response to trickle infection of 4,000 *O.circumcincta* day^{-1} was comparable to that observed in young sheep with no previous worm experience and exposed to the same rate of trickle infection (Coop *et al.* 1977; Sykes and Coop, 1977). In contrast to this Anderson (1973) reported that adult sheep with previous experience of infection exhibited pepsinogen levels higher than those of younger sheep. Thus, in addressing the results of the present work it would seem prudent (if not a little parochial) to consider only previous studies which utilised animals of the same age, physiological state and undergoing similar infection regimes.

In the work of McAnulty (1990) serum pepsinogen levels of ewes, also trickle infected at 4,000 *O.circumcincta* larvae day^{-1}, from four weeks prior to parturition, peaked at around 400 μm l^{-1} at parturition and remained at this level until the end of the trial period four weeks later. Pepsinogen concentrations of uninfected control ewes remained at around 100 μm l^{-1} throughout. These results were reflected in significantly higher FECs of infected compared with control ewes *viz.* approximately 500 epg at parturition in infected ewes and zero in non infected. In the present study the significantly higher FECs of LE ewes compared to HE ewes (360 and 30 epg, respectively), in the week preceding parturition, were not paralleled by concentrations of plasma pepsinogen *viz.* - 450 and 340 μm l^{-1} for HE and LE ewes respectively; although these levels were of similar magnitude to those reported by McAnulty (1990).

In conclusion, despite significant variation in LW and CS of ewes, resulting from differential energy supply, a breakdown of resistance to parasitic

challenge was experienced by both nutritional treatment groups. Differences in body condition at parturition, achieved through differential energy supply during late pregnancy, appeared to have no significant effect on the timing of this breakdown or the subsequent re-establishment of resistance during lactation.

Chapter 4

Level of infection and current nutritional plane

4.1 Introduction

The timing and magnitude of the periparturient breakdown in resistance appears to vary between years. Reporting specifically on *H. contortus*, Gibbs and Barger (1986) found that FECs increased in late pregnancy and peaked at 4,000 epg at the start of lambing. Similarly, Brunsdon and Vlassoff (1971) observed a rise in egg counts (predominantly of *H.contortus*) immediately prior to lambing but reaching a mean peak of 795 epg, approximately 6 weeks into lactation. In earlier studies Brunsdon (1970, 1971) had observed a peak of 1,200 epg seven weeks after lambing (Brunsdon, 1970) and 1,700 epg, eight weeks post partum (Brunsdon, 1971). It is unclear what factors influence this variation.

One theoretical possibility may be the variation in larval challenge experienced by grazing ewes. Sheep systems in New Zealand, involving high stocking rates and intensive, rotational grazing, result in high levels of pasture larval contamination and subsequent larval challenge to grazing stock. Growing lambs which elicit negative production responses *viz.* reduced LW gain, when exposed to such challenge, tend to receive preferential treatment and are grazed on 'clean pasture`. Adult ewes however are likely to be subjected to higher larval challenge, particularly in the autumn, as they are used to 'clean-up' pasture contaminated by young stock in spring and summer (Brunsdon *et al.*, 1986). The larval intake of ewes can be calculated to range from almost zero to well in excess of 200,000 larvae day^{-1} using the data of Vlassoff (1982), as outlined in Chapter 3, and assuming a dry matter intake of 1.2 kg ewe^{-1} day^{-1}, reflecting the high rate of pasture utilisation normally imposed by pregnancy.

The nutritional state of the host may be a further source of variation. Given the mounting evidence that the resistance to parasitic infection in the growing lamb is sensitive to host nutrition and in particular protein supply (Bown *et al.*, 1991; Coop *et al.*, 1995; van Houtert *et al.*, 1995) it is plausible that during late pregnancy and early lactation, a period when the requirement for metabolisable protein (MP) relative to ME is high, responses to parasitic infection may be sensitive to nutrient supply.

The objective of this trial was to determine whether a combination of low feed intake and high larval challenge during late pregnancy would precipitate breakdown of resistance to parasitic infection.

4.2 Materials and methods

Experimental design

Forty eight mixed age twin-bearing Coopworth sheep (LW = 64 ± 5.0 kg) were allocated to one of four treatment groups balanced for LW twelve weeks prior to parturition. One group (n=12) was fed in excess of their estimated ME requirement and the remainder below requirement (n=36). From eight weeks prior to parturition all groups were trickle infected with *T. circumcincta* infective larvae. The low plane sheep were dosed with either 5,000 (L5, n=12), 10,000 (L10, n=12), or 20,000 (L20, n=12) larvae day^{-1}, while the high plane group was dosed with 10,000 (H10) larvae day^{-1}. Larval dosing ceased at parturition at which time all sheep were slaughtered.

Feeding and management

From twelve to nine weeks prior to parturition, group H10 grazed ryegrass/white clover pasture with mass of approximately 800 kg DM ha^{-1}. Groups L5, L10 and L20 grazed similar pasture of lower mass (400 - 600kg DM ha^{-1}) and restricted in area. All sheep were offered 200g day^{-1} concentrate pellet

(Table 4.1). Crude protein determination was based on Kjeldahl extraction and ME content of forages and concentrate was estimated using the equations of Barber *et al.* (1984) and Alderman (1985), respectively. These were outlined in Chapter 3.

Table 4.1 Composition and analysis of pellets and hay offered to sheep during Trial 2 (g kg^{-1} DM)

	Feed	
	Pellet[1]	Lucerne hay
Composition		
Barley grain	468	
Bran/Pollard	460	
Molasses	8	
$CaCO_2$	30	
NaCl/Selenium premix	34	
Analysis		
Dry matter	875	850
DOM[2]	882	488
Crude protein	144	168
M/D[3]	10.2[4]	8.0[5]

[1] *APR Plus*, Target Stockfeeds, Archers Milling Co. Rangiora, NZ
[2] DOM Digestible organic matter
[3] MJ ME kg^{-1} DM
[4] Estimated per Alderman (1985)
[5] Estimated per Barber *et al.* (1984)

Nine weeks prior to parturition the sheep were drenched and brought indoors into individual pens and offered the pelleted concentrate and chopped lucerne hay (Table 4.1). The H10 sheep were initially offered 1,000 g day^{-1} of hay and 200 g day^{-1} pellets, the latter increasing to 1,000 g day^{-1} at parturition. In terms of ME offered this approximated to 8.6 MJ ME $head^{-1}$ day^{-1} at housing,

increasing to 15.7 MJ ME head^{-1} day^{-1} by parturition. The L groups were initially offered 500 g day^{-1} of hay and 200 g day^{-1} pellets, the hay increasing to 600 g day^{-1} and the pellets to 700 g day^{-1} by parturition. ME offered to the L groups at housing was approximately 5.2 MJ head^{-1} day^{-1} and increased to approximately 10.3 MJ ME head^{-1} day^{-1} by parturition. Concentrate ration was increased by 200g day^{-1} if BHB concentration exceeded 1.5 mmol l^{-1} in L group sheep. The additional DM and ME provided by this supplement was averaged across all sheep in the L group for inclusion in DM and ME intake data (Table 4.2). The calculation of additional DM and ME offered to L group sheep is outlined in Appendix 4.1. Levels of additional DM and ME offered are presented in Appendix 4.1.1. Total feed refusals of H10 and L groups were recorded daily and mean weekly DM and ME intake was calculated for the two groups from six weeks prior to parturition for estimation of intake (Table 4.2). The method of calculating DM and ME of refusals and the levels recorded are presented in Appendices 4.2 and 4.2.1., respectively.

General methodology

Live weight was recorded weekly from twelve until one week prior to parturition. Body condition score was determined on weeks 11 and ten and then weekly from eight weeks until one week before lambing. Condition score was determined by the same operator on each occasion as described in Chapter 3. Blood samples were collected twice weekly from housing (nine weeks prior to lambing) until parturition. The methods of collection, centrifugation and storage of plasma were as described in Chapter 3. Plasma BHB was again determined twice weekly to monitor energy status (Koch and Feldbruegge, 1987). The birth weight of lambs was recorded and summed for each ewe as soon as practical after parturition for determination of litter weight. Curved crown-rump length was measured with a flexible tape across the dorsum from the forehead (midway between the eyes) to the tail head using the method of

Joubert (1956). Chest circumference was measured with a flexible tape around the widest point of the thoracic cavity.

Parasitology

All parasitological techniques were identical to those outlined in Chapter 3. Faecal samples were obtained twice weekly from two weeks after the commencement of the trickle infection *viz.* from 6 weeks prior to parturition. Plasma pepsinogen was measured twice weekly, as described in Chapter 3, from 35 days prior to and until parturition by the method of Edward *et al.* (1960) and Berghen (1987).

Statistical analysis

Statistical analysis of results was identical to the methods outlined in Chapter 3, above.

4.3 Results

Calculated mean feed intake of the groups during the six weeks prior to parturition is shown in Table 4.2. Feed intake did not appear to be influenced by parasite challenge and there appeared to be no reduction in intake due to uterus/rumen compression. The provision of additional concentrate ration in response to elevated BHB concentration was limited to one or two individuals within each of the three L groups. This did not appear to be influenced by parasite challenge. Levels of additional DM and ME offered are presented in Appendix 4.1.1.

Live weight and body condition score

Live weight and CS are shown in Figures 4.1 and 4.2, respectively. Both changed significantly with time ($P<0.01$). There was a time by nutrition interaction in both LW and CS ($P<0.01$) due to a significantly greater change in H10 than L sheep. Live weight gain tended to decrease as the level of infection increased within the L groups *viz.* 5.9 ± 0.46, 5.1 ± 0.70, and 4.5 ± 0.61 kg for groups L5, L10 and L20 respectively, but this effect was not significant.

Table 4.2 Mean daily dry matter (DM) and metabolisable energy (ME) intake of High & Low plane groups in the final six weeks of pregnancy in Trial 2

	High Plane		Low Plane					
Week prior to parturition	DM intake (kg day^{-1})	ME intake (MJ day^{-1})	DM intake (kg day^{-1})	ME intake (MJ day^{-1})	DM intake (kg day^{-1})	ME intake (MJ day^{-1})	DM intake (kg day^{-1})	ME intake (MJ day^{-1})
			L5		**L10**		**L20**	
6	1.55 ± 0.003	13.9 ± 0.02	0.92 ± 0.012	8.5 ± 0.14	0.93 ± 0.014	8.6 ± 0.09	0.92 ± 0.014	8.5 ± 0.10
5	1.53 ± 0.010	13.7 ± 0.09	0.94 ± 0.012	8.7 ± 0.13	0.92 ± 0.013	8.5 ± 0.12	0.93 ± 0.015	8.6 ± 0.14
4	1.69 ± 0.018	15.4 ± 0.16	0.92 ± 0.010	8.5 ± 0.14	0.93 ± 0.014	8.6 ± 0.11	0.94 ± 0.012	8.7 ± 0.12
3	1.69 ± 0.024	15.4 ± 0.22	0.94 ± 0.014	8.7 ± 0.09	0.95 ± 0.009	8.8 ± 0.04	0.96 ± 0.009	8.9 ± 0.13
2	1.72 ± 0.007	15.6 ± 0.06	1.11 ± 0.005	10.3 ± 0.14	1.13 ± 0.004	10.4 ± 0.09	1.12 ± 0.003	10.4 ± 0.02
1	1.71 ± 0.014	15.6 ± 0.13	1.12 ± 0.006	10.4 ± 0.12	1.13 ± 0.004	10.4 ± 0.01	1.13 ± 0.004	10.4 ± 0.04

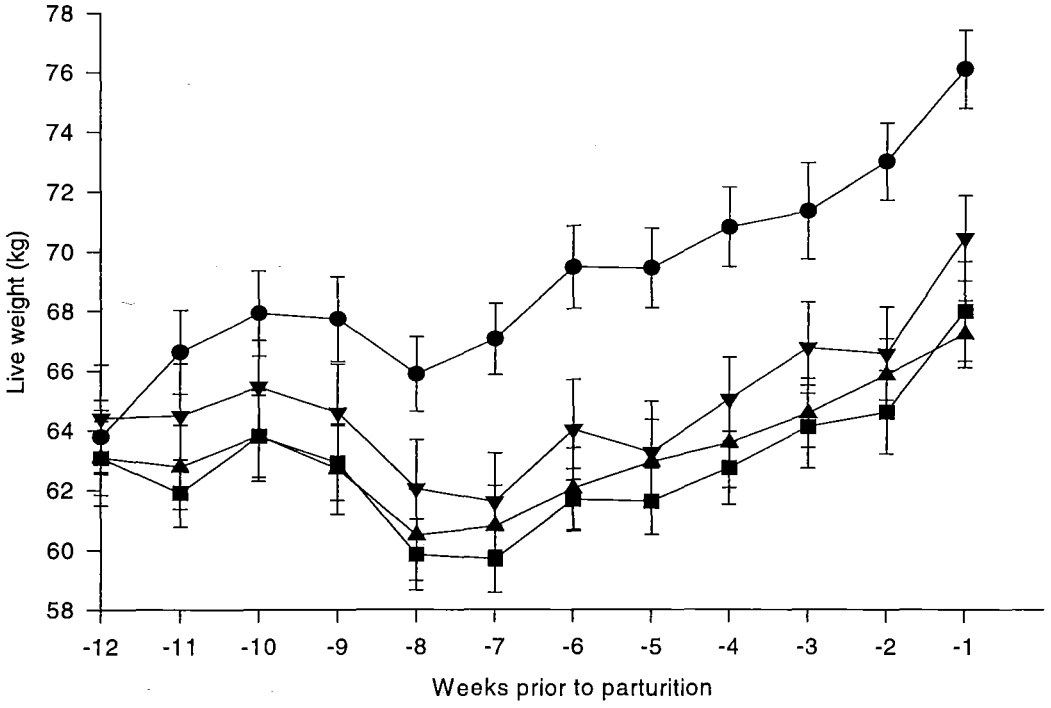

Figure 4.1 Mean live weight of ewes in treatment groups H10 (●) L5 (▼) L10 (■) and L20 (▲) prior to parturition in Trial 2

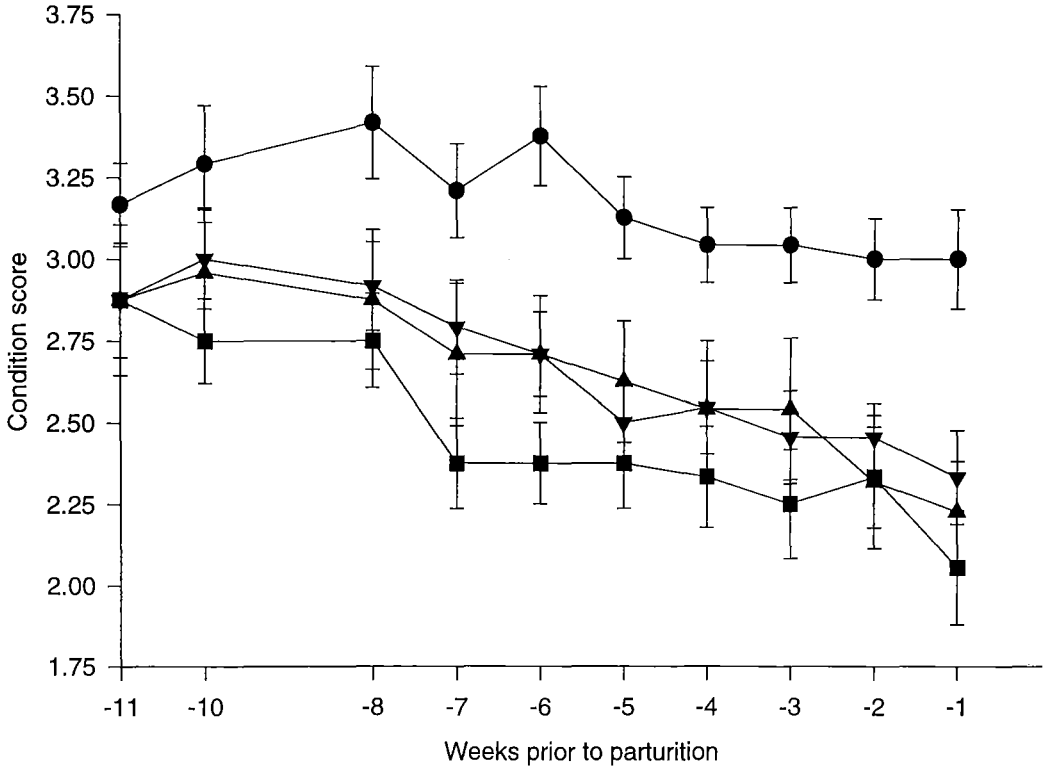

Figure 4.2 Mean condition score of ewes in treatment groups H10 (●) L5 (▼) L10 (■) and L20 (▲) prior to parturitionin Trial 2

Lambing performance is shown in Table 4.3. The litter weight of ewes in the H group at 9.3 ± 0.66 kg was significantly greater than that of L group ewes at 8.0 ± 0.30 ($P<0.05$). There was no effect of level of infection on ewe litter weight. Curved crown-rump length averaged 45.9 ± 1.22 cm for lambs born to H sheep and 45.4 ± 1.00 cm for lambs born to L sheep (NS). Chest circumference averaged 33.0 ± 0.76 in lambs born to both H and L sheep (Table 4.3). Curved crown-rump length and chest circumference were unaffected by level of infection.

Table 4.3 Mean litter weight, curved crown-rump length and chest circumference (± SEM) of lambs at birth inTrial 2

Group	Litter weight (kg)	Mean crown to rump length (cm)	Chest circumference (cm)
H10	9.3 ± 0.66	45.9 ± 1.22	33.0 ± 0.83
L5	8.2 ± 0.70	46.0 ± 0.86	32.7 ± 0.77
L10	7.6 ± 0.39	45.1 ± 1.07	33.2 ± 0.77
L20	8.1 ± 0.50	45.1 ± 1.07	33.0 ± 0.68

Parasitology

Faecal egg counts increased with time in all groups ($P<0.01$) but remained below 50 epg until two weeks before lambing when counts in the four groups increased rapidly (Figure 4. 3).

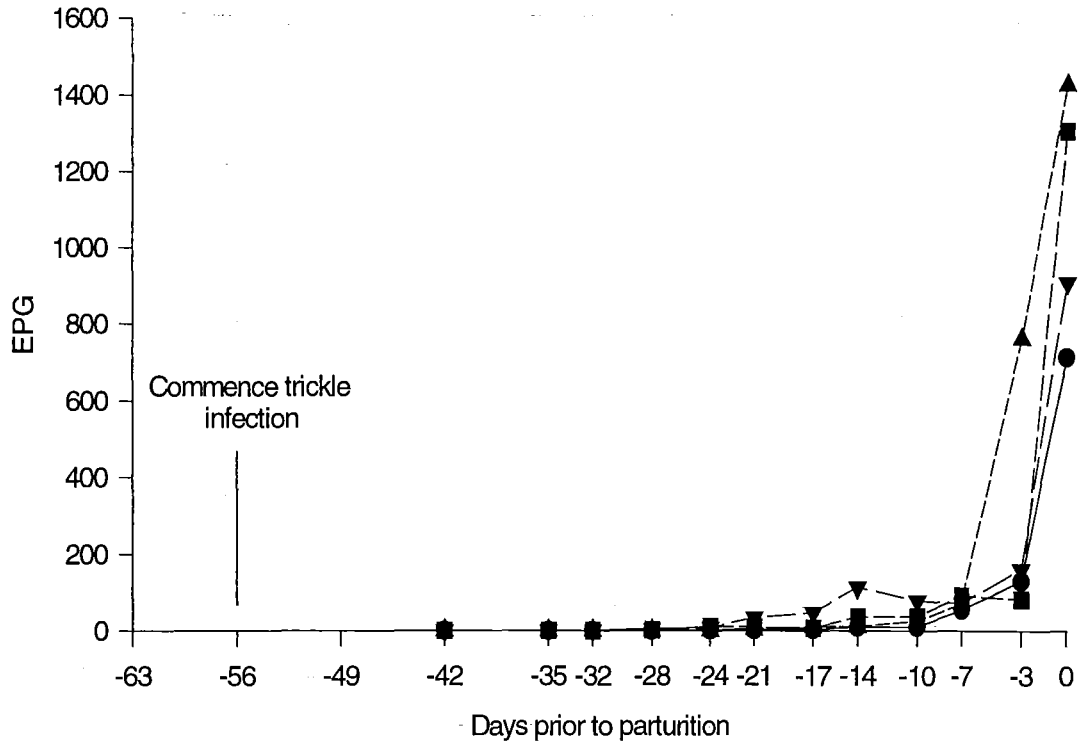

Figure 4.3 Geometric mean (Log_{10} (count+1)) faecal egg counts (EPG) of sheep in groups H10 (●) L5 (▼) L10 (■) and L20 (▲) prior to parturition in Trial 2

At parturition sheep in the H10 group had the lowest faecal egg count - *viz.* 710 epg - but this level was not significantly different from sheep in the L groups. As the level of infection increased there was a tendency for egg count to increase - *viz.* 900, 1,300 and 1,430 epg in groups L5, L10 and L20 respectively, at parturition, but again these differences were not statistically significant (P=0.08).

Worm burdens are shown in Table 4.4. Worm burdens were lower in H10 than in L10 sheep, at the same level of larval challenge - *viz.* 5680 vs 8050 - but this difference was not significant. Increased larval challenge resulted in higher worm burdens at slaughter - *viz.* 4,700, 8,050, 11,590 in groups L5, L10 and L20 respectively, but again these counts were not significantly different. Very low levels of fourth stage larvae were recovered from abomasa - again these tended to be lower in H10 than L10 sheep and tended to increase with increasing level of larval challenge. These effects were not significant. Worm burdens

recovered following the digestion of abomasa were also relatively low and followed the trend of greater burdens with lower nutrient supply and increasing larval challenge but these were again not significant.

Plasma pepsinogen concentration increased significantly with time ($P<0.01$) but there was no time by nutritional treatment effect and no time by infection level effect (Figure 4.4).

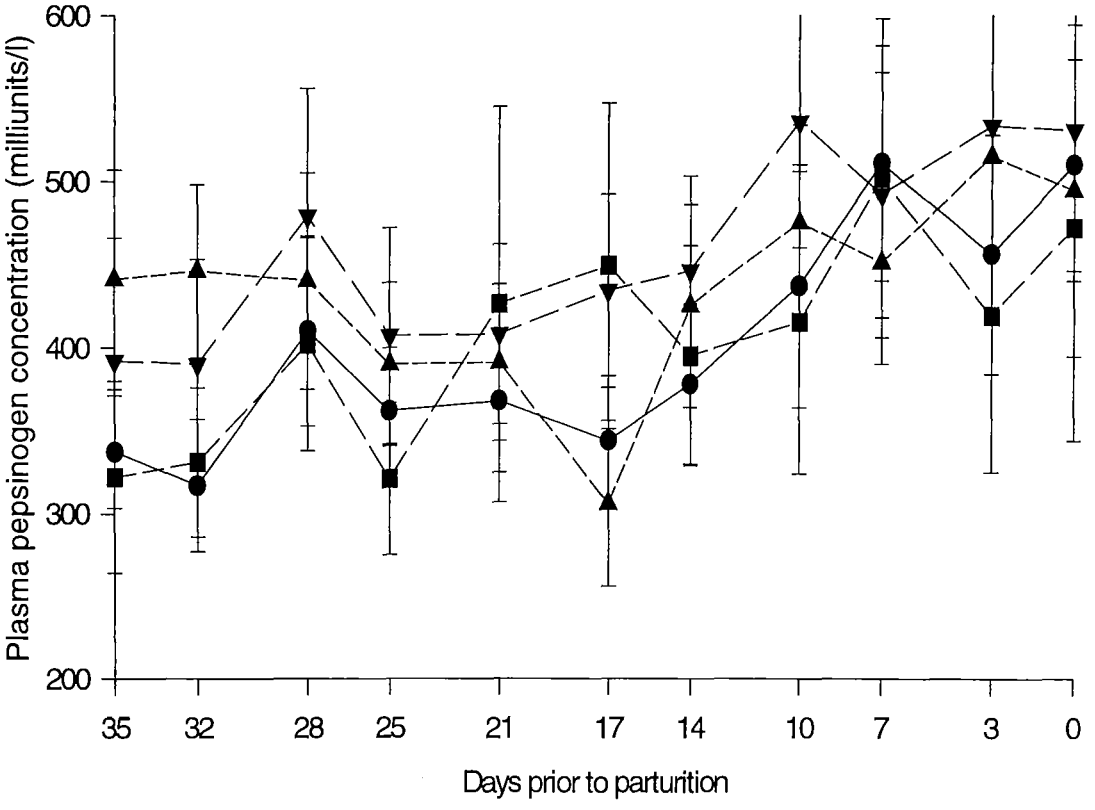

Figure 4.4 Mean plasma pepsinogen concentrations of H10 (●) L5 (▼) L10 (■) and L20 (▲) sheep prior to parturition in Trial 2

Table 4.4 Geometric mean (log_{10} (count+1) worm burden (range) of sheep at parturition sorted by developmental stage and gender from abomasal wash and digest in Trial 2

Group	L4♀	L4♂	Digest (L4)	L5♀	L5♂	Total*
H10	3 (0-440)	1 (0-330)	26 (0-742)	3405 (660-10890)	1996 (440-5500)	5680
L5	7 (0-1650)	1 (0-1650)	43 (0-762)	2507 (330-13420)	1703 (110-8470)	4700
L10	30 (0-9240)	22 (0-6820)	64 (0-259)	3986 (550-31240)	2685 (880-22220)	8050
L20	46 (0-660)	7 (0-770)	81 (12-1163)	6479 (1430-21890)	4599 (1760-17050)	11590

* Totals differ from sum of individual developmental stage and gender counts due to logarithmic transformation

4.4 Discussion

The results presented above confirm those of the previous trial (Chapter 3) that energy supply *per se* appears to be of little importance in the periparturient breakdown of resistance to GI parasitism. Additionally, the results tend to suggest that a combination of low energy provision and high nematode larval challenge are unlikely to precipitate the breakdown. Despite differences in LW of 7 kg and 0.8 of a CS between high and low plane sheep, all groups exhibited a rise in FECs in the week immediately preceding parturition. The timing of the rise in egg output was similar to that reported by Brunsdon and Vlassoff (1971), Gibbs and Barger (1986), and the previous trial outlined in Chapter 3.

Given the limitation on animal numbers the design of the trial was weighted to providing most information on the effect of high larval intake at low feeding levels. Despite a trend towards increased faecal egg count with increasing larval challenge, emerging in the week prior to parturition, FECs were not significantly different between infection rate groups. This lack of effect of larval challenge on FECs tends to disagree with the assumption of McSporran and Andrewes (1988) that elevated counts observed in autumn lambing ewes related directly to the greater level of pasture contamination in the autumn compared with spring.

In general, the relationship between FECs and larval challenge does not appear to be particularly clear cut. Symons *et al.* (1981) studied the interrelationship between the level of exposure of lambs to *O. circumcincta* and various parasitological and metabolic responses. They found that lambs on a dosing regime of 12,000 larvae per week had the highest FECs (1,050 epg) while those receiving 37,500 and 120,000 per week had egg counts below 100 epg of faeces for most of the experiment. There was no explanation given for this trend. However, production was significantly depressed in lambs on the higher levels of infection *viz.* 37,500 and 120,000 week^{-1} suggesting that a higher larval

challenge may have reached a threshold level sufficient to stimulate an immune response to the detriment of production. In the present study this did not appear to be the case as sheep on the highest level of larval challenge (20,000 larvae day^{-1}) appeared to be no more susceptible to infection (as manifested by faecal egg output) than sheep on the lower larval challenge levels. There was however, a trend toward decreased LW with increased infection rate, perhaps indicating that production was slightly compromised due to an immune response. *In utero* egg count data may have been useful in accounting for the lack of significance between FECs. There is evidence to suggest that eggs per female worm may be influenced by the level of larval challenge or subsequent parasite crowding in the gut (Chiejina and Sewell, 1974a; Coop *et al.*, 1977).

One could speculate that if the periparturient breakdown was related to the elevated nutritional demands encountered by sheep in late pregnancy and early lactation, then animals on a lower plane of nutrition would experience a nutrient deficit at an earlier stage than an animal on a higher plane of nutrition. As a result of this, low plane animals might be expected to display a rise in egg output sooner than those on a higher nutrient plane. Clearly this has not been the case in either Trial 1 or Trial 2. This does not, however, rule out the possibility that an even more severe energy deficit may indeed precipitate an earlier breakdown of resistance.

Despite the lack of significant effects of energy supply and/or larval dose rate on worm burdens (Table 4.4), results obtained were of interest. The predominance of adult worms over larval stages was perhaps surprising since the trickle infection continued until the point of slaughter. One of the mechanisms by which the periparturient breakdown is believed to occur is a relaxation of the animal's ability to reject incoming larvae (Connan, 1968; O'Sullivan and Donald, 1970). The very low numbers of juvenile larvae recovered at slaughter would suggest that a very large proportion of the incoming larvae were indeed being rejected. However, the presence of adult

worms would tend to indicate that, at some point during the eight week infection period, there had been a relaxation in the animals' ability to reject incoming larvae. It could also be argued that the presence of high numbers of adult worms, at parturition, may simply reflect a gradual build up of infection having occurred throughout the eight week infection period. It is unlikely that even in adult, resistant sheep, prevention of establishment of ingested larvae would be complete (Leathwick *et al.*, 1997). This may be further supported by the data for L4 larvae, where, although overall numbers were low, ranges tended to indicate the presence of substantial numbers of juvenile worms in some animals on the low plane of nutrition.

In a study by McAnulty *et al.* (1991) twin bearing Coopworth ewes were given a single challenge infection of 20,000 *T. circumcincta* larvae, 4 weeks prior to lambing, and slaughtered 21 days later. The establishment rate of this challenge infection can be calculated to be approximately 6%. In the present trial then, if we assume (unrealistically perhaps) complete resistance until six weeks prior to parturition, followed by a 6% establishment rate until parturition, then the three levels of larval challenge might be expected to have resulted in burdens approximating 12,600, 25,200 and 50,400 for groups L5, L10 and L20, respectively. There will of course be a death rate to consider since there is a continual turnover of the adult worm population. Reports of estimated death rate of *T. circumcincta* in adult sheep vary. Leathwick *et al.* (1997) calculated the average death rate of *T. circumcincta* in Romney ewes averaged over a six week period, nine weeks after lambing to be 10.6%, and from the work of Jackson *et al.* (1988) calculated a death rate of 5.4% in sheep between 42 and 28 days prior to lambing. The mean worm burdens obtained in the present work of 4,700, 8,050 and 11,590 for groups L5, L10 and L20, respectively tend to indicate that establishment and/or death rate of worms varied with the level of infection. There may well be an association between either of these immune responses

since both worm size and fecundity are known to be reduced with increasing larval challenge (Krupp, 1961; Coop *et al.*, 1977).

It could also be argued that adult worms had, in part at least, originated from previously arrested larvae - which had resumed development due to some unknown trigger associated with parturition (Dunsmore, 1965; Brunsdon 1966; Connan 1968). The anthelmintic drench at housing would have eliminated arrested larvae present at that time but burdens may well have built up again during the course of the trickle infection. Without serial slaughter information it is impossible to predict to what extent arrestment of larvae was involved in the immune response of these animals. Worm burdens identified as L4 stage and those recovered following digestion of abomasa were very few in number and this would tend to suggest that at the time of slaughter either the immune mechanism resulting in arrestment of larvae had been suspended or that inhibition had not been an attribute of this strain of parasite.

The lack of effect of larval dose rate on plasma pepsinogen concentration was perhaps unexpected and difficult to explain, since it has been shown that even in resistant animals plasma pepsinogen levels increase with larval challenge (Anderson, 1973; Reid and Armour, 1975). Pepsinogen concentrations in the present study were relatively low, peaking at approximately 540 mu l^{-1} plasma. Leyva *et al.* (1982) reported an elevation in plasma pepsinogen concentration following daily infection of 6,000 *O. circumcincta,* in the range of 800-1,000 mu l^{-1} plasma. This, they claimed was comparable to the rate and extent of elevation observed in naive lambs exposed to similar rates of infection. The low levels of plasma pepsinogen concentration observed in the present work were of similar magnitude to those obtained in the previous trial.

In conclusion results from this trial suggest that a combination of low energy provision and a high nematode larval challenge are unlikely to significantly affect the periparturient parasite status of ewes.

Chapter 5

The effect of metabolisable energy and protein supply on the periparturient breakdown in single and twin bearing sheep

5.1 Introduction

The development of resistance in young animals to GI parasitism and the pathogenicity of infection have been demonstrated to be influenced by host nutrition (Gibson, 1963). More specifically, it was shown by Bown *et al.* (1991) that protein supply, rather than the energy, was responsible for the development of resistance.

Protein supplementation may provide the nutrient resources required to repair gut tissue, damaged by GI parasites, thus enhancing host resilience to infection. Additionally, protein may specifically enhance the resistance of the host to infection. The precise mechanisms by which this might occur are unclear at present. However, in immunocompetant animals the acquired immune response is dependent on the presence of lymphocytes (Gill *et al.*, 1994) and the production of effector substances from the parasitised gut mucosa (Miller, 1984). Many of the components which appear to be involved in immunocompetancy such as leucotrienes, immunoglobulins and mucoproteins are proteinaceous in nature and the responses observed to protein supplementation may reflect an overcoming of a parasite induced protein deficiency, particularly in young growing animals. In a recent study by Kambara *et al.* (1993) protein supplementation responses were demonstrated to be more effective in promoting resistance in 2-6 month old sheep than in 7-12 month old sheep. This may be related to the greater MP demand relative to ME demand in the very young lamb *viz.* 11 g MP MJ^{-1} ME at 10 kg LW compared to 6.3 g MP MJ^{-1} ME at 40 kg LW (Ørskov, 1992). In a review of the implications of nutrition for the ability of ruminants to withstand GI nematode infections,

van Houtert and Sykes (1996) commented on this and indicated that the periparturient ewe experiences similar requirements for MP relative to ME - in the order of 7 - 8 g MP MJ^{-1} ME - at a time when host resistance is known to be compromised. Robinson (1990) discussed the nutrient requirements of multiple bearing ewes in late pregnancy and pointed out that amino acid requirements at this time are well in excess of those supplied by high quality forage. This would tend to suggest that multi parous ewes may experience competition for available nutrients between maintaining an effective immune response and for the demands of late pregnancy and early lactation.

The present study was undertaken to assess the relative importance of ME and MP supply on the periparturient parasite and immunological status of ewes. Single and twin bearing ewes were compared to determine the influence of pregnancy status on the magnitude of the periparturient breakdown.

5.2 Materials and methods

Experimental design

From a mob of one hundred female Coopworth sheep (age range 3-6 years), which had been oestrus synchronised and mated seventy two days previously, thirty two single-bearing (LW = 49 ± 3.2 kg) and thirty two twin-bearing (LW = 51 ± 2.8 kg) individuals were identified by ultra sound scanner (Aloka Echo camera, model SSD 210DXII. Probe 3.5 MHz external, model UST-5021). Within these groups animals were assigned hierarchically according to LW and CS to four nutritional treatments in a 2x2 factorial design for energy (E) and protein (P) supply. The sheep were trickle infected with 10,000 *T. circumcincta* and 7,000 *T. colubriformis* infective larvae day^{-1} from seven weeks before and until parturition and were slaughtered three weeks later.

Feeding and Housing

The sheep were drenched and housed nine weeks prior to parturition in individual pens on slatted floors. Low (E1) and high (E2) energy diets consisted of lucerne hay and pelleted grain concentrate (Table 5.1) in the ratios 70:30 and 30:70, respectively, and were offered at levels designed to promote 0 and +50g day^{-1} gain in maternal bodyweight according to AFRC (1993). Low protein (P1) and high protein (P2) diets contained either 0 or 80g kg^{-1} DM added fishmeal, to provide approximately 120 and 200g CP kg^{-1} DM, respectively. To achieve these dietary regimes three pelleted rations were formulated (Table 5.1). Pellet 1 was used in E1P1 and E2P1 rations varying according to the ratios stated above. Pellet 2 was used in E1P2 rations and Pellet 3 was used in E2P2 rations. *In vitro* digestibility of organic matter of the feed was estimated using the cellulase/pepsin method of Jones and Hayward (1975). Metabolisable energy content of forages and concentrates was estimated using the formulae of Barber *et al.* (1984) and Alderman (1985), respectively, as outlined in Chapter 3. Crude protein content was calculated as outlined in Chapter 3. E1 and E2 diets, respectively, supplied between 10.7 and 13.2 MJ of ME day^{-1} to single-bearing and between 12.9 and 15.4 MJ ME day^{-1} to twin-bearing sheep. P1 and P2 treatments were estimated to supply MP at the rate of 94 and 110g day^{-1} to single bearing and 113 and 133g day^{-1}, to twin-bearing sheep. During lactation the treatments were maintained. A milk yield of 2.0 and 3.0 kg day^{-1} for single and twin-bearing sheep, respectively, was assumed, and E1 and E2 levels were designed to supply respectively, 19 and 23 MJ ME day^{-1} to single and 28 and 32 MJ ME day^{-1} to twin-bearing sheep. The objective was to achieve a weight loss during lactation of 100 and 0g day^{-1}, for treatments E1 and E2, respectively. Individual animal rations were formulated based on the ME and MP requirement tables of AFRC (1993). From these data, regression equations incorporating individual LW of each animal were used to calculate daily ME and MP requirements during pregnancy and during lactation (See Appendices 5.1.1- 5.1.3). Calculation of ME and MP content of rations is outlined in Appendices 5.2.1 and 5.2.2, respectively. An average rumen outflow rate of 0.05

hour^{-1} was assumed for the duration of the trial period as this is the estimated outflow rate for animals fed at two times maintenance ME requirement – the approximate level of feeding for ewes in late pregnancy. Feed supply for each sheep was adjusted weekly according to LW. Feed refusals were recorded daily for estimation of intake. Dry matter concentration of feed refusals was assumed to be the same as that of feed offered. Mean CP intake during pregnancy and lactation is given in Appendix 5.3.1 and 5.3.2, respectively.

Table 5.1 Composition and analysis of lucerne hay & concentrate pellets offered to sheep during Trial 3 (g kg^{-1} DM)

	Feed			
	Pellet 1	Pellet 2	Pellet 3	Lucerne Hay
Composition				
Barley	500	375	462	
Maize	500	675	462	
Fishmeal		250	75	
Analysis				
Dry Matter	850	850	850	880
DOM[1]	987	958	995	691
Crude Protein	116	260	196	178
M/D[2]	14.0	13.5	13.8	9.3
FME[3]	13.3	12.3	12.8	8.9
UP5[4]	14.0	88.0	31.0	34.0
RP5[5]	105.0	147.0	99.0	131.0

[1]DOM, digestible organic matter
[2]MJ ME kg^{-1} DM
[3]FME Fermentable ME of diet (AFRC, 1993)
[4]UP5 Undegradable protein content at mean rumen digesta fractional outflow rate of 0.05/h. (AFRC, 1993)
[5]RP5 Degradable protein content at mean rumen digesta fractional outflow rate of 0.05/h. (AFRC, 1993)

General Methodology

Ewe LW was recorded weekly from nine weeks prior to and until parturition. Body condition score was assessed in weeks 9, 7, 4 and 1 prior to parturition by the same operator as outlined in Chapter 3. Lamb birth weight was recorded as soon as practical after parturition. Lambing performance was measured and analysed as litter weight ie. birth weight of single born lambs and the sum of birth weight of twin born lambs. Lamb body weight at 'weaning' was recorded and lamb growth rate for the 21 day lactation was calculated.

Parasitology

Prior to housing the ewes had been grazed at the Lincoln University research farm. Parasitic larval challenge for the six month period prior to housing was estimated as outlined in Appendix 2 of Chapter 3. All parasitological techniques were similar to those described in Chapter 3. *T.colubriformis* infective larvae (strain LIU/89-1) were obtained from AgResearch, Ruakura Research Centre, Hamilton, New Zealand and had been passaged through naive lambs several times. At necropsy, approximately 10 metres of small intestine, distal to the pylorus, was tied off and removed for determination of worm burden (on small intestinal washing only). Additionally, a 5 cm section of the eviscerated small intestine, 30 cm from the pylorus was excised and opened. Mucus was gently scraped off with a microscope slide as described by Douch *et al.* (1983) and placed in a 1.5 ml eppendorf tube. This was immediately immersed in liquid nitrogen for two minutes and then stored at - 80° C until used in a larval migration inhibition (LMI) assay, as outlined below. The remainder of the eviscerated small intestine was gently flushed with tap water and rinsed contents collected in a beaker. The contents were sieved and made up to approximately 200 ml in 10% formalin as outlined for abomasal contents in Chapter 3. Abomasal tissue was obtained as outlined previously and abomasal and small intestinal worm burdens were determined as described in Chapter 3.

Measurement of worm length

Mean worm length was determined to establish whether stunting of worm development was a feature of any immune response observed. Twenty adult male and twenty adult female *Teladorsagia* spp. worms were isolated, at random, for each animal, from the formalin preserved worm burden samples, obtained at slaughter. Each worm was placed on a few drops of tap water on a microscope slide onto which a cover slip was carefully placed. The mounted worm was then video taped using a stereo microscope (Olympus SZH stereo microscope) with video port. Images were calibrated by use of a 1 mm stage micrometer. The recorded worm images were then projected onto a screen and the length of the worm determined by tracing a map wheel along the worm image. Actual worm length was calculated by length of worm image X magnification factor.

In utero egg counts

A further 20 female spp. were obtained from the preserved abomasal sample, isolated from each animal. These were soaked for 24 hours in lactophenol to clear the specimens before in utero egg counts were undertaken using a compound binocular microscope. Individual worms were placed in a droplet of tap water on a microscope slide onto which a cover slip was placed. Individual eggs within the reproductive tract were recorded.

Lymphocyte blastogenesis test (LBT)

Six twin bearing ewes from each of the four treatment groups were randomly selected for the *in vitro* measurement of lymphocyte responses to parasite antigen and mitogens specific for thymic derived (T) and bursa derived (B) lymphocytes. A modification of the method of Kambara *et al.* (1993) was adopted. In summary, 10 ml of blood was collected in EDTA K3 vacuum blood collection tubes (Venoject, Terumo Medical Corporation, Elkton, MD, USA) at seven, six, four, and one week before parturition and at three weeks post

partum. In addition the abomasal lymph nodes were aseptically dissected at slaughter. Peripheral lymphocytes were removed from blood and lymph nodes by density-gradient centrifugation in 60% lymphoprep (Life Technologies, Penrose, Auckland, NZ). The cell suspension was adjusted to a concentration of 2.3 x 10^6 lymphocytes ml^{-1} in Dulbecco's Modified Eagle Media (DMEM) (Difco, Detroit, USA) containing 10% Foetal Bovine Calf Serum (FBCS) with benzylpenicillin and streptomycin sulphate (Gibco Ltd, Gaithersburg, MD, USA).

Infective larval antigen was prepared from infective (L3) larvae of T.*circumcincta* and T.*colubriformis* and suspended in phosphate buffered saline before being frozen in liquid nitrogen. The larvae were then disrupted by crushing and sonic vibration and allowed to stand overnight at 4°C. The supernatant was collected, filtrated through 0.2 µm filter (MFS, Dublin, CA, USA) and kept at -20°C until required. The protein concentration of the resultant larval antigen was measured by a method modified from Lowry *et al.* (1951) and used in the LBT at 64 µg ml^{-1}.

The following mitogens were also used in the LBT: Concanavalin A (Con A), (Sigma, St. Louis, MO, USA) at 5 µg ml^{-1}; Protein A, (Sigma, St. Louis, MO, USA) at 20µg ml^{-1}; Lipopolysaccharide W (LPS) (Difco, Detroit, MI, USA) at 25 µg ml^{-1}; and Phytohaemagglutinin (PHA), (Difco, Detroit, MI, USA) at 12.8 µg ml^{-1}.

Two hundred µl of cell suspension and either 25 µl of buffer as a control or 25 µl of mitogen or infective larval antigen were cultured in triplicate in U-bottom micro-test plates (Nunc. Naperville, IL., USA). These were incubated for 48 hours in a humidified atmosphere, followed by a further 20 hours culturing with 1µCi per well of tritiated thymidine (TRA 61; Amersham, Australia). Cells were harvested onto filter paper discs using a cell harvester (Cambridge

Technology, Watertown, MA, USA), and the uptake of labelled thymidine was measured by a liquid scintillation counter (Pharmacia LKB, Uppsala, Sweden). The result was expressed as a stimulation index (SI) for each animal being the ratio of counts per minute (cpm) from each antigen/mitogen stimulated well divided by the mean cpm of unstimulated control wells (Kambara *et al.*, 1993).

Larval Migration Inhibition (LMI) Assay

A LMI assay was undertaken to detect variation in antiparasitic activity of the GI mucus of sheep from the different nutritional treatment groups. The four sub-groups of twin bearing ewes used for LBT analysis were also utilised for the LMI assay. The assay was carried out at AgResearch, Wallaceville Animal Research Centre, Upper Hutt, New Zealand, using the method of Rabel *et al.* (1994). Briefly, this determines the inhibition of larval migration through 20 μm nylon mesh sieves following incubation in a test substance (in this instance intestinal mucus) relative to control larvae incubated only in diluent. Test samples were assayed as single wells only, due to the limited volume of mucus obtained. The control was assayed in triplicate. Larval migration inhibition was determined using the formulae:

$$LMI = \frac{A - B \times 100}{A}$$

where A = number of worms migrating in control well and B = number of larvae migrating in mucous test wells, and expressed as a percentage.

Statistical Analysis

Repeated measures analysis of variance (ANOVA) was undertaken on LW, CS, FECs and cell stimulation indices using the general linear model procedure on the SYSTAT package (SYSTAT, 1990). Litter weight, weaning weight, lamb growth rate, worm burdens, worm lengths and in utero egg counts were analysed by ANOVA. Faecal egg counts, worm burdens and cell stimulation

indices were log transformed (log 10 (count + 1)) before analysis. Larval migration indices were analysed using the Kruskal-Wallis non parametric test (SYSTAT, 1990). Pearson product moment correlation coefficient was utilised to determine levels of correlation between results obtained from LBT, LMI and four parasitological parameters measured *viz.* T. *circumcincta* worm burden, T. *colubriformis* worm burden, a mean faecal egg count of the final five weeks of the trial and the faecal egg count of the sheep immediately prior to slaughter.

5.3 Results

Of the sixty-four sheep in the trial, the pregnancy status of six was incorrectly diagnosed while five aborted in the later stages of pregnancy. The sheep which had aborted returned positive results for a toxoplasma latex agglutination test. These eleven sheep were excluded from the trial. The numbers of sheep remaining in each group are given in Table 5.2. Metabolisable energy and MP intake during late pregnancy and the first two weeks of lactation are given in Tables 5.2, 5.3 and 5.4. Ration formulation ensured that the ME requirement of the animal was the first criteria to be met. In some cases, as a result of this, rations provided protein in excess of the MP requirements of AFRC. During pregnancy, single and twin bearing sheep in the P2 groups consumed, on average 78 and 94 g of fishmeal day $^{-1}$ and during lactation 146 and 203 g day $^{-1}$, respectively.

Live weight and body condition score

Mean LW of single and twin bearing sheep is given in Figures 5.1.1 and 5.1.2, respectively. There was a strong interaction between time with pregnancy status, with energy level and with protein level ($P< 0.001$) in all cases. Twin-bearing sheep gained, on average, 5.2 kg more weight than their single-bearing contemporaries. E2 sheep gained 6.0 kg more than E1 sheep and those on the P2 diets gained, on average, 2.4 kg more than those on the P1 diets. Mean LW immediately after parturition was lower for E1 sheep (52.9 ± 0.16 kg) than E2 sheep (58.2 ± 0.17 kg) ($P<0.001$). There was no effect of protein supply or pregnancy status on LW at this time.

Table 5.2 Calculated metabolisable energy (ME) intake (ME offered - ME refused) prior to parturition in Trial 3 (MJ ME day^{-1})

Group	n	Week 16	Week 17	Week 18	Week 19	Week 20	Week 21
Single							
E1P1	(8)	9.5 ± 0.08	9.5 ± 0.08	9.2 ± 0.11	9.1 ± 0.11	9.1 ± 0.11	11.7 ± 0.22
E1P2	(7)	9.4 ± 0.09	9.6 ± 0.09	9.2 ± 0.12	9.3 ± 0.12	9.2 ± 0.12	12.3 ± 0.25
E2P1	(6)	11.8 ± 0.11	12.2 ± 0.10	11.9 ± 0.14	11.8 ± 0.15	12.1 ± 0.14	15.2 ± 0.29
E2P2	(8)	11.7 ± 0.08	12.2 ± 0.08	12.0 ± 0.11	12.5 ± 0.11	12.2 ± 0.11	15.4 ± 0.25
AFRC[1]		10.0	10.0	11.2	11.2	12.8	12.8
Twin							
E1P1	(6)	10.6 ± 0.11	11.4 ± 0.10	12.0 ± 0.14	11.1 ± 0.15	11.2 ± 0.14	15.1 ± 0.29
E1P2	(6)	11.1 ± 0.11	11.6 ± 0.10	12.4 ± 0.14	11.8 ± 0.15	11.3 ± 0.14	16.7 ± 0.29
E2P1	(6)	13.3 ± 0.11	14.4 ± 0.10	15.3 ± 0.14	14.2 ± 0.15	13.9 ± 0.14	15.5 ± 0.29
E2P2	(6)	13.8 ± 0.11	14.9 ± 0.10	15.4 ± 0.14	15.1 ± 0.15	14.6 ± 0.14	18.3 ± 0.29
AFRC[2]		13.1	13.1	15.3	15.3	18.3	18.3

AFRC[1] – AFRC (1993) Recommended ME (MJ day^{-1}) requirement for 60 kg single bearing sheep at zero LW gain
AFRC[2] – AFRC (1993) Recommended ME (MJ day^{-1}) requirement for 70 kg twin bearing sheep at zero LW gain

Table 5.3 Calculated metabolisable protein (MP) intake (MP offered - MP refused) prior to parturition in Trial 3 (g MPday^{-1})

Group	n	Week 16	Week 17	Week 18	Week 19	Week 20	Week 21
Single							
E1P1	(8)	79.2 ± 0.73	79.4 ± 0.65	77.1 ± 0.82	75.8 ± 0.94	75.8 ± 0.94	96.5 ± 1.73
E1P2	(7)	97.8 ± 0.83	99.2 ± 0.74	96.0 ± 0.94	96.1 ± 1.06	95.4 ± 1.07	127.8 ± 1.98
E2P1	(6)	85.8 ± 0.97	88.8 ± 0.86	85.3 ± 1.10	84.6 ± 1.24	86.2 ± 1.25	109.9 ± 2.31
E2P2	(8)	95.8 ± 0.73	99.8 ± 0.65	97.1 ± 0.82	98.9 ± 0.93	98.9 ± 0.94	126.1 ± 1.73
AFRC[1]		84	84	90	90	98	98
Twin							
E1P1	(6)	89.3 ± 0.97	96.2 ± 0.86	101.5 ± 1.10	94.3 ± 1.24	94.9 ± 1.25	126.9 ± 2.31
E1P2	(6)	116.4 ± 0.97	122.2 ± 0.86	129.9 ± 1.10	124.2 ± 1.24	119.0 ± 1.25	176.3 ± 2.31
E2P1	(6)	98.1 ± 0.97	106.6 ± 0.86	109.3 ± 1.10	101.7 ± 1.24	100.4 ± 1.25	113.1 ± 2.31
E2P2	(6)	114.7 ± 0.97	123.8 ± 0.86	126.8 ± 1.10	123.3 ± 1.24	119.3 ± 1.25	152.9 ± 0.29
AFRC[2]		101	101	112	112	126	126

AFRC[1] - AFRC (1993) Recommended MP (g day^{-1}) requirement for 60 kg single bearing sheep at zero LW gain
AFRC[2] - AFRC (1993) Recommended MP (g day^{-1}) requirement for 70 kg twin bearing sheep at zero LW gain

Table 5.4 Calculated metabolisable energy (MJ ME day^{-1}) and metabolisable protein (g MP day^{-1}) intake (offered - refused) of sheep during lactation in Trial 3

Group	ME week 1	MP week 1	ME week 2	MP week 2
Single				
E1P1	19.0 ± 0.22	158.1 ± 1.81	18.9 ± 0.27	157.4 ± 2.25
E1P2	19.3 ± 0.25	200.0 ± 2.07	19.3 ± 0.31	200.4 ± 2.57
E2P1	23.3 ± 0.29	169.8 ± 2.41	23.5 ± 0.37	171.6 ± 3.00
E2P2	23.7 ± 0.22	193.7 ± 1.81	23.5 ± 0.27	191.6 ± 2.25
AFRC[1]	23.7	222	23.7	222
Twin				
E1P1	26.9 ± 0.29	226.6 ± 2.41	25.8 ± 0.37	216.7 ± 3.00
E1P2	28.1 ± 0.29	294.7 ± 2.41	28.1 ± 0.37	294.7 ± 3.00
E2P1	30.2 ± 0.29	223.7 ± 2.41	27.3 ± 0.37	200.4 ± 3.00
E2P2	30.0 ± 0.29	248.4 ± 2.41	31.0 ± 0.37	256.8 ± 3.00
AFRC[2]	32.2	297	32.2	297

AFRC[1] – AFRC (1993) Recommended ME (MJ day^{-1}) and MP (g day^{-1}) requirement for 60 kg sheep suckling single lamb and assuming milk yield of 2.0 kg day^{-1}
AFRC[2]– AFRC (1993) Recommended ME (MJ day^{-1}) and MP (g day^{-1}) requirement for 60 kg sheep suckling twin lambs and assuming milk yield of 3.0 kg day^{-1}

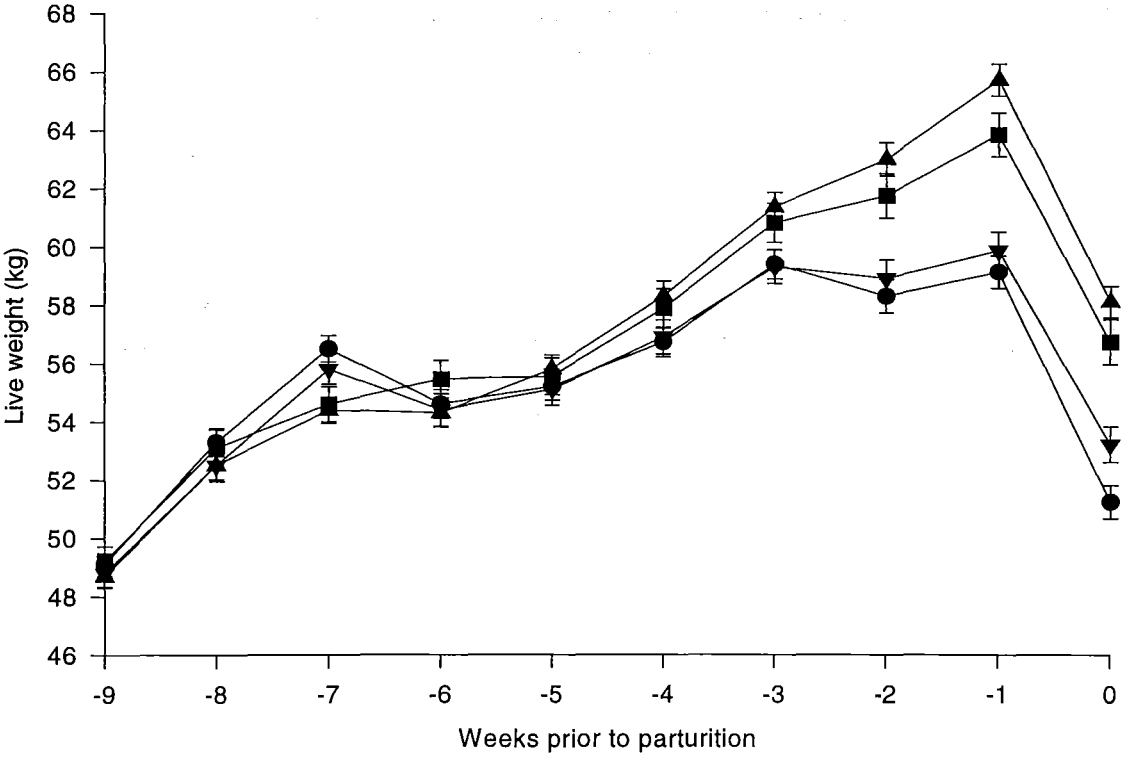

Figure 5.1.1 Mean live weight of single bearing ewes in treatment groups E1P1(●) E1P2(▼) E2P1(■) and E2P2(▲) prior to parturition in Trial 3

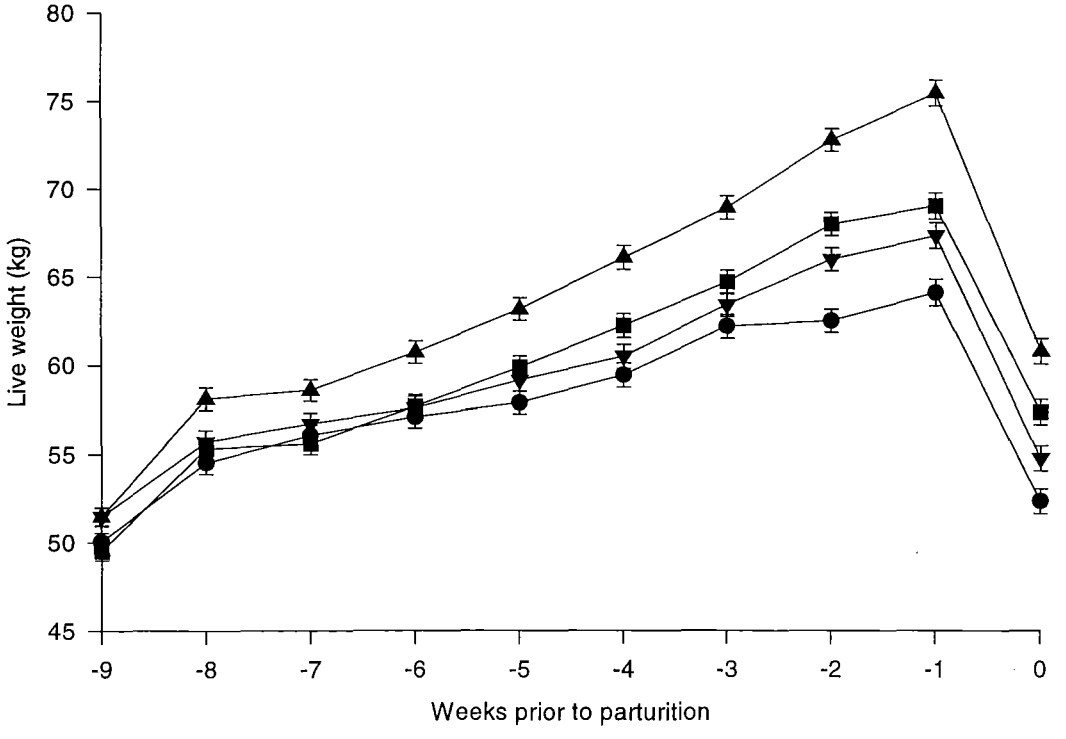

Figure 5.1.2 Mean live weight of twin bearing ewes in treatment groups E1P1(●) E1P2(▼) E2P1(■) and E2P2(▲) prior to parturition in Trial 3

Mean CS are shown in Figures 5.2.1 and 5.2.2. There were strong interactions between time and energy and protein intake on CS ($P<0.05$) but no interaction between time and pregnancy status. Mean CS decreased in all groups from nine until one week before lambing with the exception of single bearing sheep in group E2P2, which gained an average of 0.2 ± 0.07 of a CS. On average sheep in group E1 lost more body condition than E2 sheep - 0.7 vs 0.2 respectively ($P< 0.01$), while sheep in P1 groups lost more body condition than P2 sheep - 0.6 vs 0.2 ($P< 0.01$).

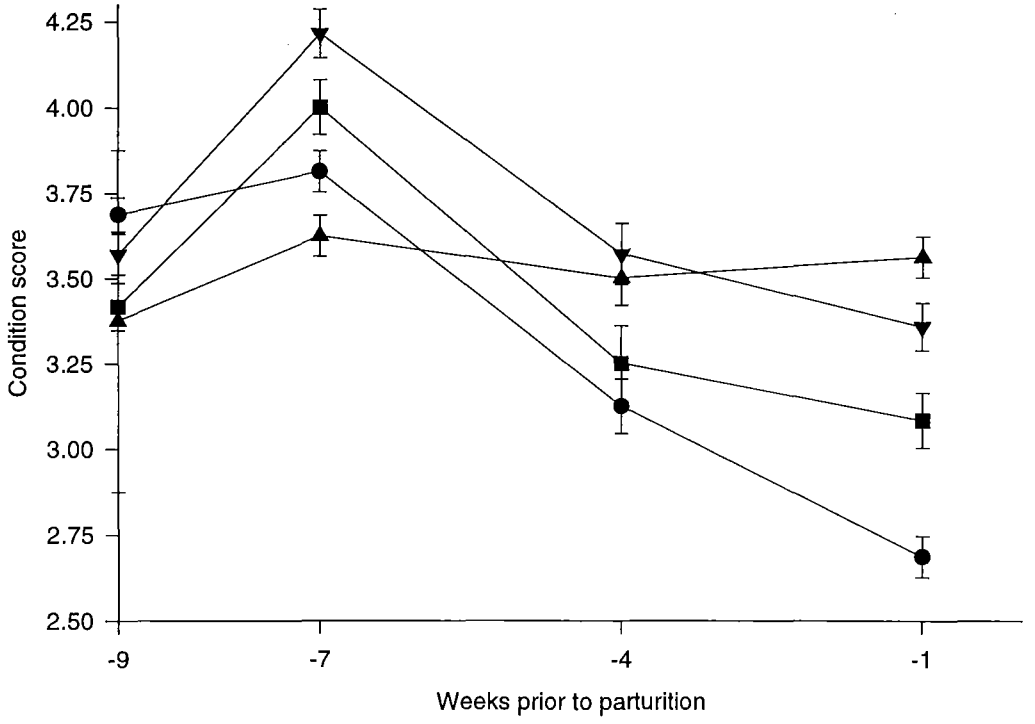

Figure 5.2.1 Mean condition score of single bearing ewes in treatment groups E1P1(●) E1P2(▼) E2P1(■) and E2P2(▲) prior to parturition in Trial 3

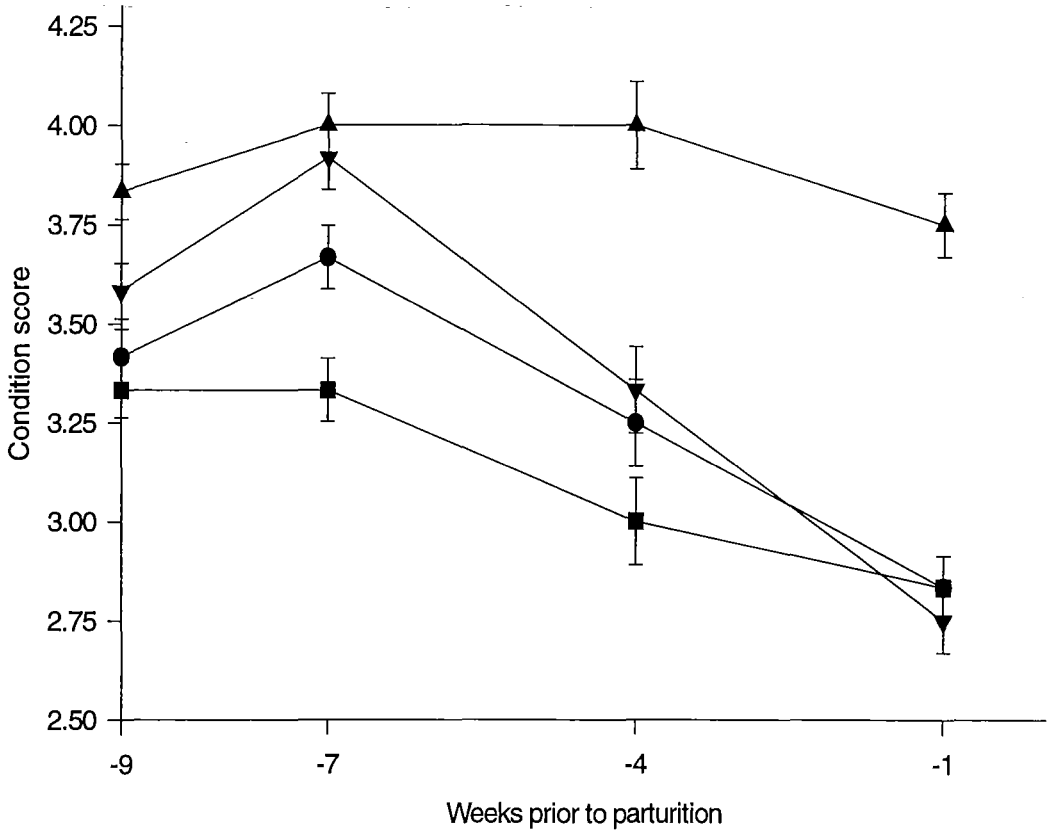

Figure 5.2.2 Mean condition score of twin bearing ewes in treatment groups E1P1(●) E1P2(▼) E2P1(■) and E2P2(▲) prior to parturition in Trial 3

Lambing performance

Litter weight of ewes in the various treatment groups are shown in Table 5.5. There was a highly significant effect of pregnancy status and energy supply on litter weight *viz.* 3.1 kg greater in twin than single bearing sheep and 0.6 kg greater in E2 than E1 sheep ($P< 0.01$ in both cases). There was also a significant effect of protein supply on litter weight ($P< 0.05$) but no interactions between pregnancy status, energy supply or protein supply. Due to an oversight the weaning weight of lambs from a number of ewes was not recorded. Table 5.6 presents re-analysed mean lamb birth weight data of the 23 ewes for which complete lamb weight data was available, together with weaning weights and the calculated lamb growth rate for the 21 day lactation period. Both rearing status and protein supply had a highly significant effect on lamb growth rate and subsequent weaning weight ($P< 0.01$).

Table 5.5 Mean litter weight (± SEM) of single and twin bearing sheep in Trial 3

	Single bearing (Kg)		Twin bearing (Kg)	
	n		n	
E1P1	8	5.05 ± 0.109^{a}	6	8.02 ± 0.146^{b}
E1P2	7	4.91 ± 0.125^{a}	6	8.47 ± 0.146^{bc}
E2P1	6	5.50 ± 0.146^{a}	6	8.12 ± 0.146^{b}
E2P2	8	6.05 ± 0.109^{a}	6	9.28 ± 0.146^{c}

Means with different superscripts in columns indicate significance ($P<0.05$)

Table 5.6 Mean individual lamb birth weights (± SEM), weaning weights (21 days post partum) (± SEM), and daily growth rate of lambs whose weight was recorded at end of Trial 3 (n = no. of ewes)

Group	n	Mean birth weight (kg)	Mean weaning weight (kg)	Growth rate g day^{-1}
Single				
E1P1	(4)	5.3 ± 0.15^{ab}	10.8 ± 0.47^{a}	264
E1P2	(4)	5.4 ± 0.17^{ab}	13.5 ± 1.03^{a}	387
E2P1	(4)	5.7 ± 0.27^{a}	12.1 ± 0.86^{a}	305
E2P2	(4)	6.4 ± 0.23^{a}	13.7 ± 0.74^{a}	346
Twin				
E1P1	(2)	3.6 ± 0.33^{c}	5.7 ± 1.05^{bc}	102
E1P2	(2)	4.2 ± 0.33^{bc}	10.2 ± 1.05^{a}	290
E2P1	(2)	4.1 ± 0.33^{bc}	6.3 ± 1.05^{b}	105
E2P2	(1)	4.6 ± 0.66^{bc}	9.8 ± 2.10^{ab}	248

Means with different superscripts in columns indicate significance ($P<0.05$)

Faecal egg counts

Eggs appeared in faeces from 28 days before lambing and subsequently increased in all groups with time ($P<0.01$), Figures 5.3.1 and 5. 3.2. There was a significant interaction between time and protein supply ($P<0.01$) and a tendency toward an interaction between time and pregnancy status ($P=0.08$) but no interaction between time and energy supply. In the period from parturition until 14 days later, FECs were significantly higher in twin than single-rearing sheep ($P<0.01$). Protein supply significantly affected faecal egg count from 21 days prior to parturition ($P<0.05$), being highly significant ($P<0.01$) from seven days before, until 21 days after lambing.

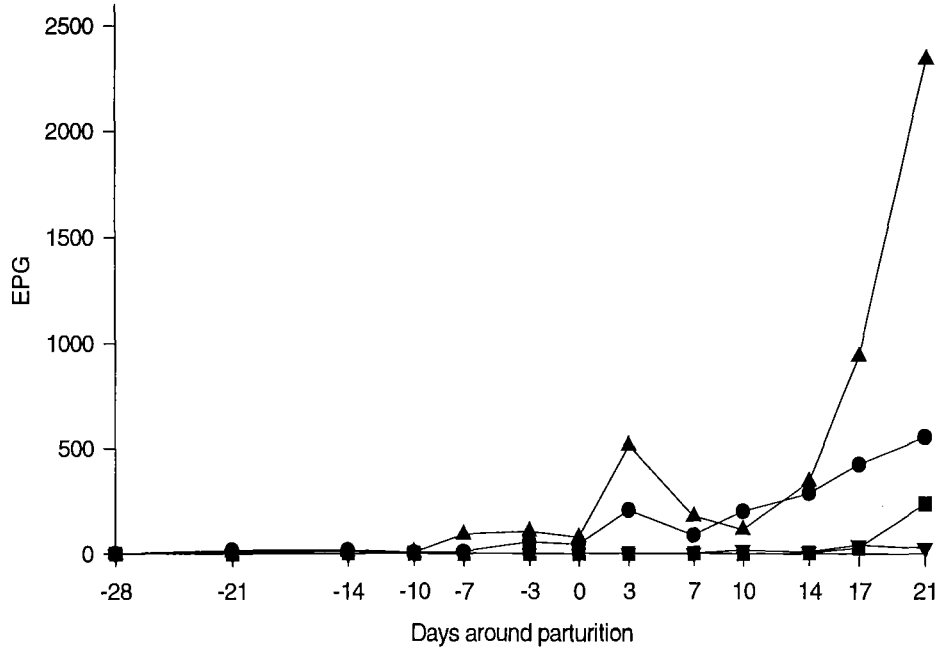

Figure 5.3.1 Geometric mean (Log_{10}(FEC+1)) faecal egg count (EPG) of single bearing sheep in groups E1P1 (●) E1P2 (■) E2P1 (▲) and E2P2 (▼) around parturition as affected by differential energy and protein supply in Trial 3

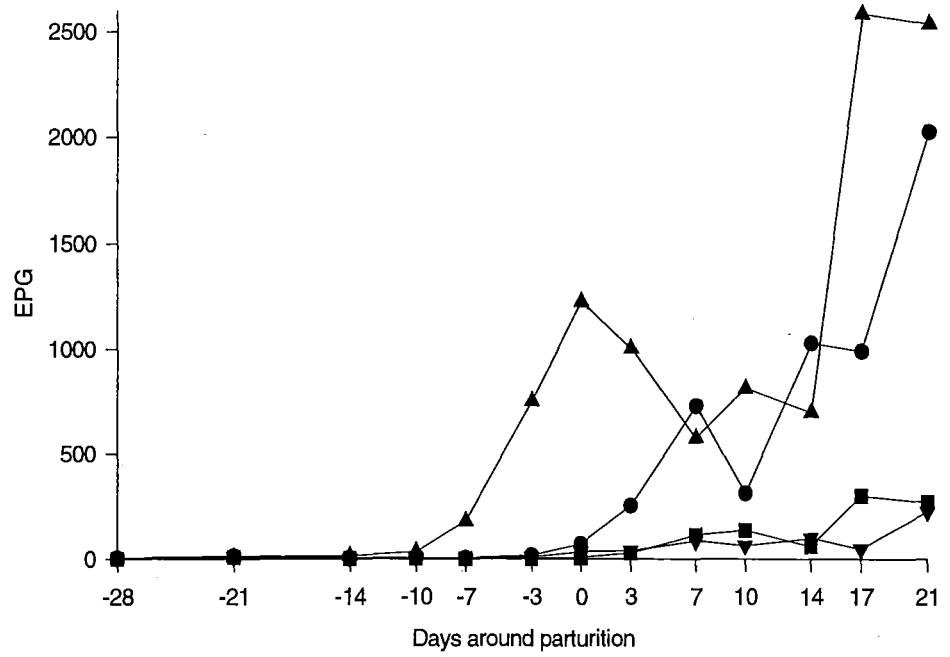

Figure 5.3.2 Geometric mean (Log_{10}(FEC+1)) faecal egg count (EPG) of twin bearing sheep in groups E1P1 (●) E1P2 (■) E2P1 (▲) and E2P2 (▼) around parturition as affected by differential energy and protein supply in Trial 3

Worm Burdens

Worm burdens are shown in Table 5.7. Mean worm burdens were 12,020 and 1,540 worms for P1 and P2 groups, respectively ($P< 0.01$). Pregnancy status also affected worm burden, with 2,290 and 8,090 worms being recovered from single- and twin- bearing and rearing sheep, respectively ($P< 0.01$). There was no effect of energy supply on worm burden. Immature worms were not found in the abomasal washings prior to digestion or in the small intestines, though a small number of L4 *Teladorsagia* spp. were recovered after digestion (Table 5.7). Worm burdens consisted predominantly of adult *Teladorsagia* spp. with very low numbers of *Trichostrongylus* spp. in all groups, except in the twin-bearing sheep offered the E2P1 diet, which harboured over 3,000 *Trichostrongylus* spp.

T.circumcincta length and in utero egg counts

T.circumcincta worm length and in utero egg count data are shown in Table 5.8. Overall female worms averaged 9.5 ± 0.17 mm while male worms averaged

7.2 ± 0.05 mm. Pregnancy status and host nutrition had no effect on worm length and there were no interactions.

In utero egg counts tended to be higher in worms from ewes which had produced a single lamb than from those producing twins *viz.* on average 44 eggs in single and 39 eggs in twin bearing ewes ($P = 0.055$). Worms from E1 group ewes had significantly greater numbers of eggs *in utero* than did worms from E2 group ewes ($P < 0.01$). There was no effect of protein supply on *in utero* egg counts and there were no interactions between treatments.

Table 5.7 Geometric mean (Log_{10} (count + 1)) worm burdens (range) of single and twin bearing sheep three weeks post partum in Trial 3

	Single bearing			Twin bearing		
	T.colubriformis	*T.circumcincta*		*T.colubriformis*	*T.circumcincta*	
	L5	L4	L5	L5	L4	L5
E1P1	172[a] (0-4290)	30[a] (0-130)	6713[a] (550-16070)	288[b] (0-4070)	70[a] (10-410)	11116[a] (6260-26420)
E1P2	1[b] (0-440)	8[a] (0-8)	578[b] (0-6050)	7[ab] (0-2750)	64[a] (10-660)	3326[a] (110-15520)
E2P1	90[ab] (0-5490)	18[a] (0-560)	9225[a] (2970-38020)	3051[a] (110-11780)	112[a] (10-840)	13090[a] (10780-16830)
E2P2	3[ab] (0-190)	11[a] (0-70)	458[bc] (0-6710)	140[ab] (0-4950)	59[a] (10-300)	3539[a] (990-8360)

Means with different superscripts within columns indicate significance ($P<0.05$)

Table 5.8 Mean worm lengths and *in utero* egg counts of *T.circumcincta* recovered from single and twin bearing ewes in Trial 3

	Single bearing			Twin bearing		
	Worm length		Eggs *in utero*	Worm length		Eggs *in utero*
	Female (mm)	Male (mm)		Female (mm)	Male (mm)	
E1P1	9.3 ± 0.13	7.3 ± 0.05	48.2 ± 1.48	9.6 ± 0.15	7.3 ± 0.06	42.3 ± 1.56
E1P2	9.4 ± 0.15	7.4 ± 0.06	53.6 ± 1.58	9.4 ± 0.17	7.3 ± 0.06	41.4 ± 1.71
E2P1	9.8 ± 0.15	7.3 ± 0.06	42.0± 1.56	9.6 ± 0.15	7.0 ± 0.06	36.8 ± 1.56
E2P2	9.9 ± 0.13	7.1 ± 0.05	33.8 ± 1.35	8.8 ± 0.15	7.0 ± 0.06	34.6 ± 1.71

Lymphocyte blastogenesis test

Mean stimulation indices (SI) of lymphocytes obtained from peripheral blood during the course of the trial are shown in Figures 5. 4.1 - 5.4.6. Repeated measures analysis indicated that lymphocyte SI changed over time in response to Con A and LPS mitogen and *Trichostrongylus* spp. antigen ($P < 0.05$). There was no effect of nutritional treatment on peripheral blood SI. Stimulation of lymphocytes obtained from the abomasal lymph node at necropsy are shown in Figure 5.5. There was no effect of nutritional treatment on lymph node SI.

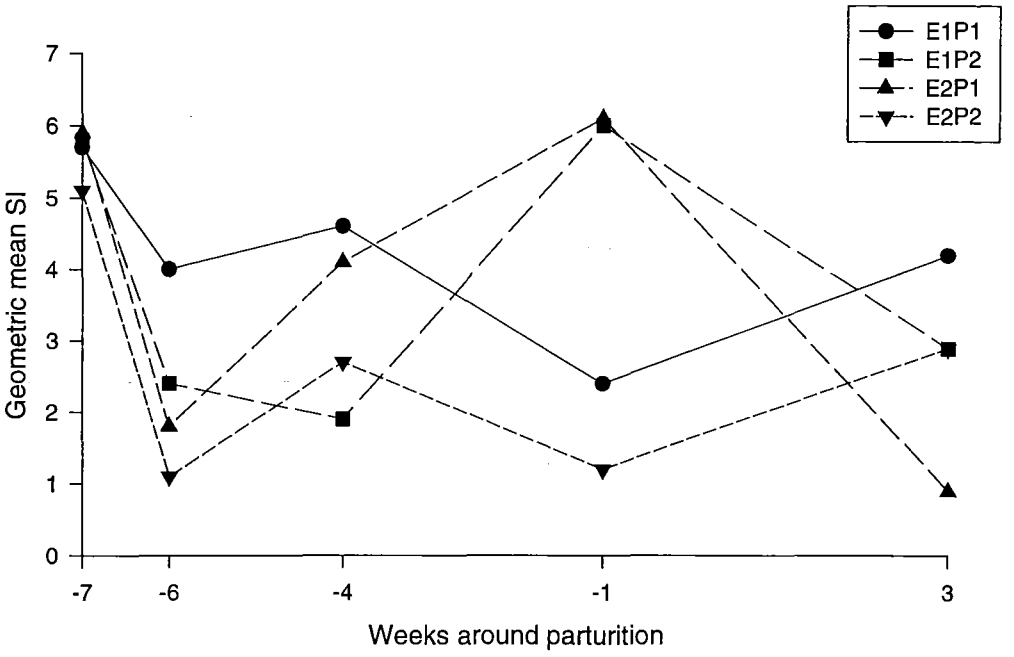

Figure 5.4.1 Geometric mean (count $\log_{10}$) stimulation indices (SI) of lymphocytes recovered from peripheral blood of ewes and cultured with concanavalin A

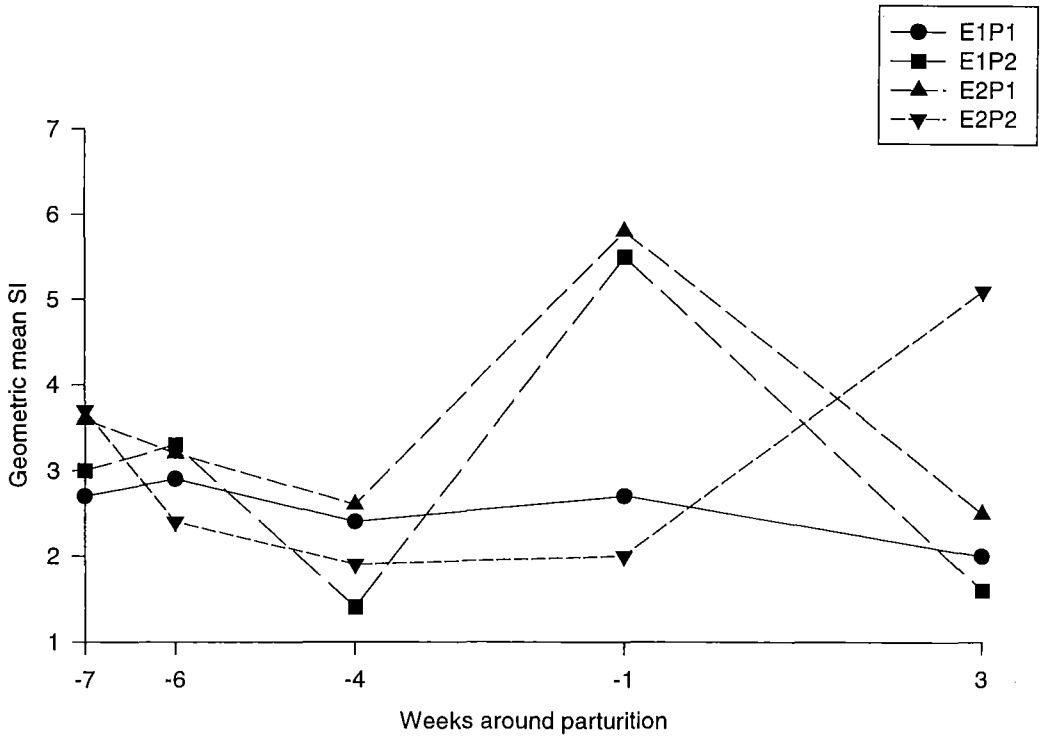

Figure 5.4.2 Geometric mean (count $\log_{10}$) stimulation indices (SI) of lymphocytes recovered from peripheral blood of ewes and cultured with *T.circumcincta* third-stage larval antigen

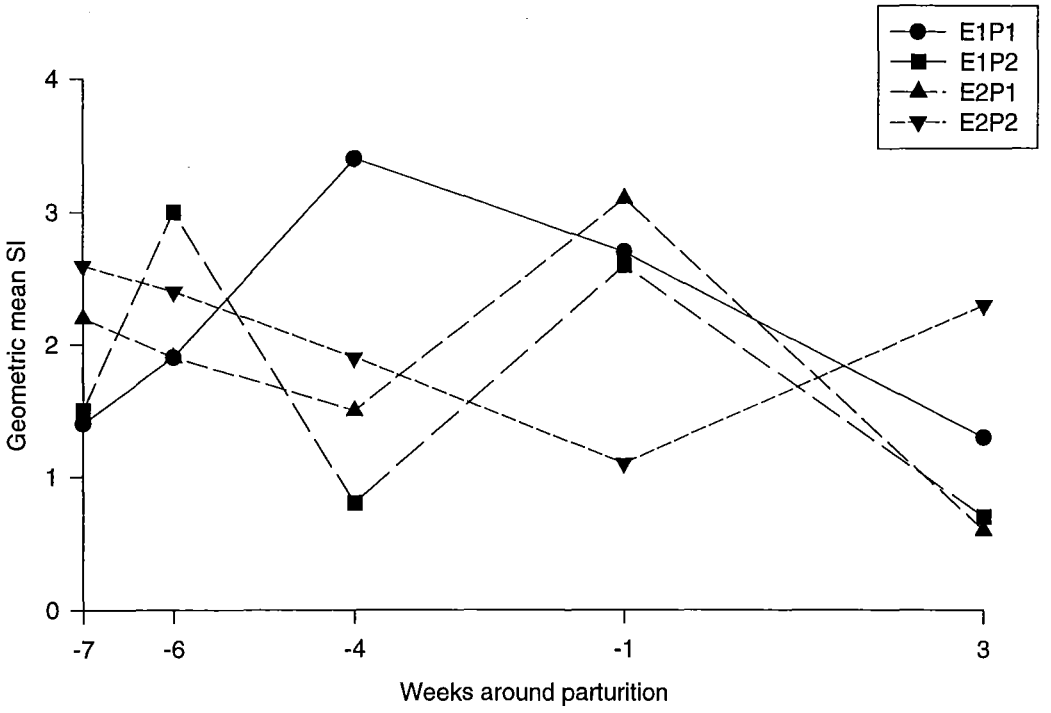

Figure 5.4.3 Geometric mean (count $\log_{10}$) stimulation indices (SI) of lymphocytes recovered from peripheral blood of ewes and cultured with *T.colubriformis* third-stage larval antigen

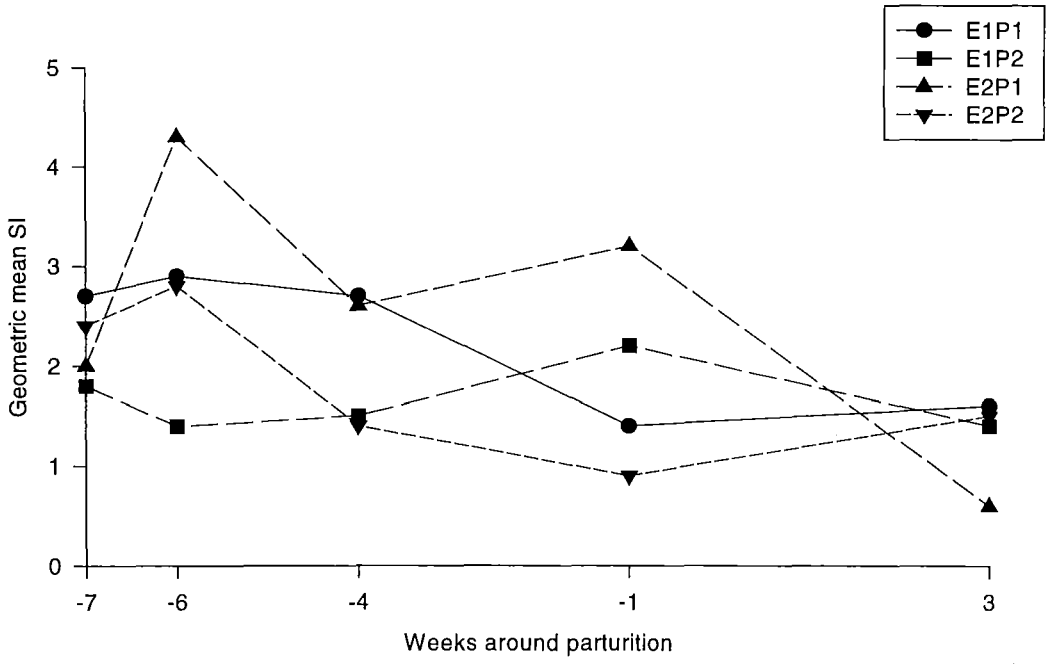

Figure 5.4.4 Geometric mean (count $\log_{10}$) stimulation indices (SI) of lymphocytes recovered from peripheral blood of ewes and cultured with Protein A

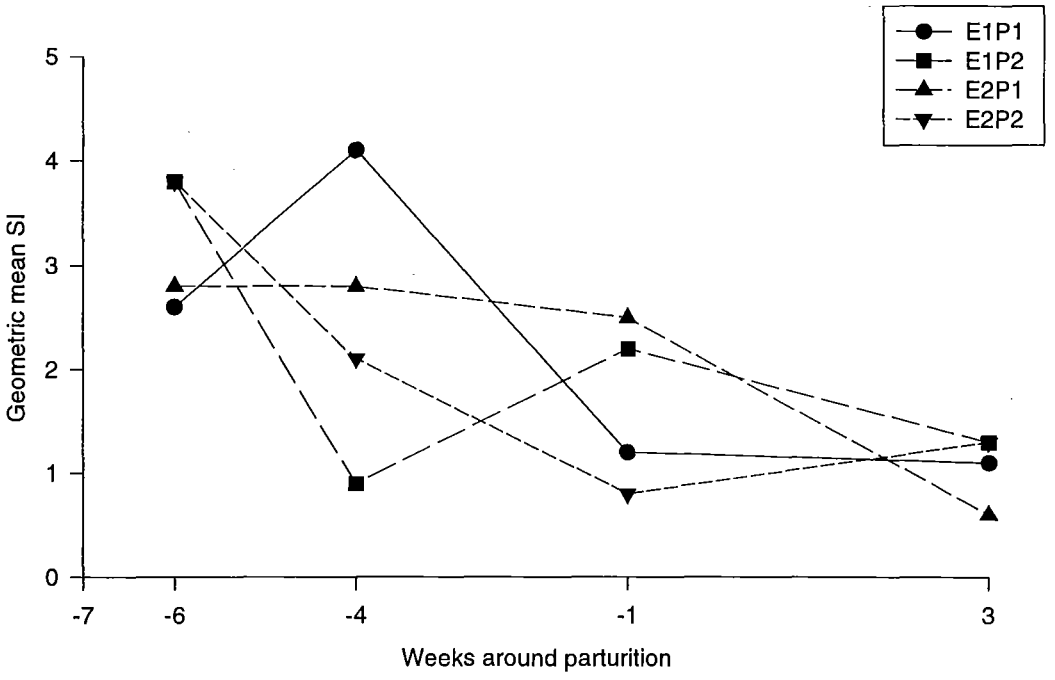

Figure 5.4.5 Geometric mean (count $\log_{10}$)stimulation indices (SI) of lymphocytes recovered from peripheral blood of ewes and cultured with lipopolysaccharide (LPS)

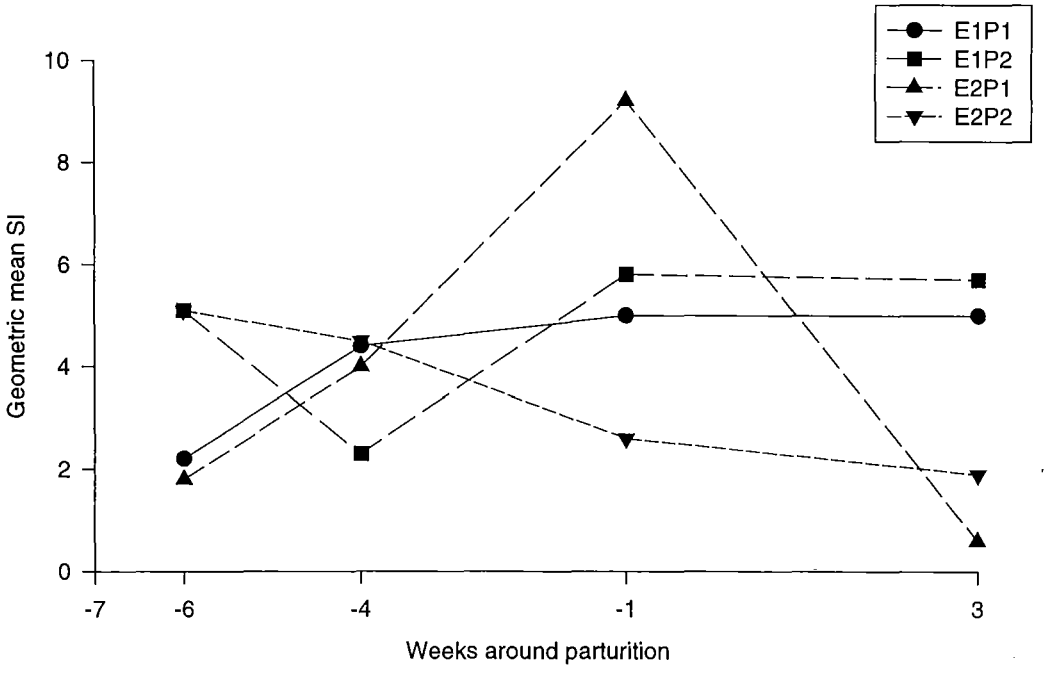

Figure 5.4.6 Geometric mean (count $\log_{10}$) stimulation indices (SI) of lymphocytes recovered from peripheral blood of ewes and cultured with phytohaemagglutinin (PHA)

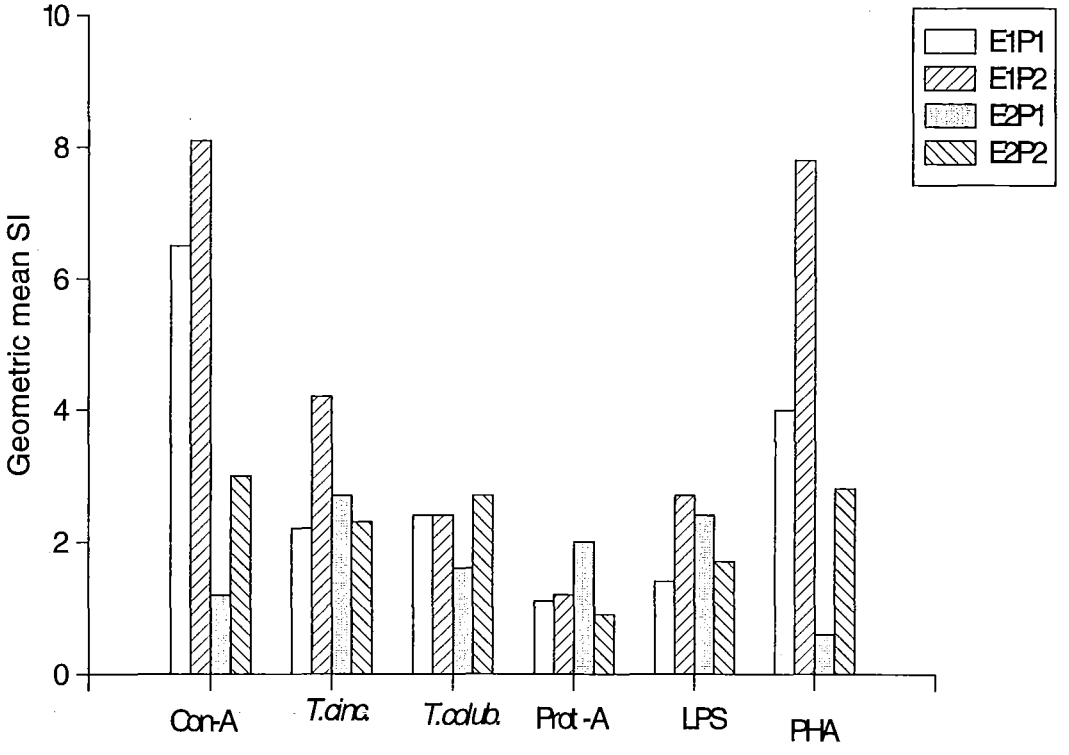

Figure 5.5 Geometric mean (count log_{10}) stimulation indices (SI) of lymphocytes recovered from the abomasal lymph node

Cell stimulation indices from peripheral blood and from the abomasal lymph nodes tended to be poorly correlated with the parasitological parameters measured as shown in Tables 5.9.1 (peripheral blood) and 5.9.2 (abomasal lymph nodes). Generally correlations between SI and parasitological results had an r value of below ± 0.5. Correlations between SI and parasitological parameters tended not to be not statistically significant ($P>0.05$) but exceptions are marked on tables.

Table 5.9.1 Correlations (Pearson) between cell stimulation indices (SI) from peripheral blood at time of slaughter and parasitological parameters measured in Trial 3 (n = 16 observations)

Mitogen/Antigen*	Con A	*T.circ.*	*T.colub.*	Prot A.	LPS.	PHA.
Parasitological measurement						
T.circumcincta worm burden	-0.023	0.203	0.187	0.289	0.024	0.150
T.colubriformis worm burden	0.288	0.470	0.502*	0.393	0.434	0.287
5 Week mean faecal egg count	0.159	0.320	0.350	0.560*	0.171	0.278
Slaughter faecal egg count	0.172	0.337	0.437	0.408	0.237	0.244

*Indicates statistically significant correlation ($P<0.05$)

Table 5.9.2 Correlations (Pearson) between cell stimulation indices (SI) from abomasal lymph node at time of slaughter and parasitological parameters measured in Trial 3 (n = 16 observations)

Mitogen/Antigen*	Con A	*T.circ.*	*T.colub*	Prot A.	LPS.	PHA.
Parasitological Measurement						
T.circumcincta worm burden	-0.156	0.002	0.078	0.176	-0.101	-0.101
T.colubriformis worm burden	-0.225	0.020	-0.456	-0.147	-0.244	-0.393
5 Week mean faecal egg count	-0.111	0.209	0.093	0.179	-0.038	-0.097
Slaughter faecal egg count	0.362	0.231	0.192	-0.086	0.028	0.015

Nb. No statistically significant correlations ($P>0.05$)
*Con A - Concanavalin A mitogen Prot A. - Protein A mitogen
T.circ. - T.*circumcincta* infective larval antigen LPS. - Lipopolysaccharide W mitogen
T.colub. - T.*colubriformis* infective larval antigen PHA. - Phytohaemagglutinin mitogen

Larval Migration Inhibition (LMI) Assay

Results obtained from the LMI assay are shown in Table 5.10. There was no effect of nutritional treatment on larval migration. Correlations between LMI and parasitological parameters are shown in Table 5.11. All correlations had an r value of less than 0.3 and were not statistically significant (P>0.05). Correlations were also calculated between LBT and LMI results (Table 5.12). It appeared that there may have been an association between T cell mitogens (Con A and PHA) and LMI trends *viz.* r values of 0.30 and 0.45 for Con A /LMI and 0.60 and 0.45 for PHA/LMI (Table 5. 12).

Table 5.10 Larval migration indices (LMI) from small intestinal mucus samples

Group/ID No.	% LMI
E1P1	
447	59.7
451	9.5
461	2.9
487	-11.2
E1P2	
438	-9.1
439	44.4
448	28.0
458	37.8
E2P1	
443	4.0
446	-3.6
449	17.1
E2P2	
444	-13.4
468	1.9
486	-20.0
488	21.5
491	14.9

Table 5.11 Correlation (Pearson) between larval migration indices (LMI) and parasitological parameters measured in Trial 3 (n=16 observations)

Parasitological measurement	Correlation
T.circumcincta worm burden	0.051
T.colubriformis worm burden	-0.145
Five week mean faecal egg count	0.118
Slaughter faecal egg count	0.268

Nb. No statistically significant correlations (P>0.05)

Table 5.12 Correlation (Pearson) between larval migration indices (LMI) and cell stimulation (SI) indices of abomasal lymph node (LBT Lymph) and peripheral blood (LBT Per Blood) at time of slaughter (n=16 observations)

	Mitogen/Antigen[a]					
	Con A	*T.circ.*	*T.colub.*	Prot A.	LPS.	PHA.
LBT (Lymph)	0.321	0.031	-0.144	0.043	-0.049	0.596*
LBT (Per Blood)	0.454	-0.063	0.101	0.008	0.340	0.452

*Indicates statistically significant correlation (P<0.05)

[a]Con A - Concanavalin A mitogen
T.circ. - T.circumcincta infective larval antigen
T.colub. - T.colubriformis infective larval antigen
Prot A. - Protein A mitogen
LPS. - Lipopolysaccharide W mitogen
PHA. - Phytohaemagglutinin mitogen

5.4 Discussion

Results from this trial clearly indicate that the periparturient breakdown in resistance can be modified by nutrient provision. More specifically it suggests that the maintenance of an effective immune response to parasitic infection at this time is more sensitive to MP than to ME supply. In addition, it demonstrates that multi-parous ewes encounter a greater degree of breakdown than single bearing ewes, possibly reflecting the greater likelihood of protein deficiency experienced by such stock in late pregnancy and early lactation.

Results presented in Figures 5.3.1 and 5.3.2 of FECs and in Table 5.7 for worm burdens tend to indicate an increasing level of parasitism with increased energy supply. For example L5 T. *circumcincta* worm burdens in twin bearing/rearing ewes were found to be 11,116 and 13,090 for E1P1 and E2P1 groups, respectively. This may however, have been a reflection of the slightly increased protein provision to animals in the lower energy groups *viz.* 217 g and 200 g of MP in the second week of lactation to sheep in groups E1P1 and E2P1, respectively, which had occurred as a result of formulating rations to primarily meet ME requirements. The effect of energy supply on parasite status was not statistically significant.

In interpreting results from nutrition/parasite interaction studies care must be taken to distinguish between current nutrient intake effects and effects resulting from the nutrient status or body tissue reserves of the animal. This study confirms the previous findings that the periparturient breakdown can occur in sheep of relatively good body condition and on an adequate current plane of nutrition in terms of ME supply - *viz.* an average CS at parturition of 3.2 and 3.0 for single and twin bearing ewes, respectively, and fed at ME levels of between 10.7 and 13.2 MJ ME day^{-1} to single bearing and between 12.9 and 15.4 MJ ME day^{-1} to twin bearing sheep. These levels can be predicted to meet animal requirements during the periparturient period (AFRC, 1993). It should also be

noted from this study that the response to supplementation in terms of FECs and worm burdens occurred where MP was supplied at a level some 6% above the predicted requirements of AFRC (1993) suggesting that nutrient requirements for immune competence may exceed those for tissue synthesis during the periparturient period. It is not possible to determine from this work whether the results reflect a response to MP supplementation per se or to the provision of a specific amino acid or other dietary component of fishmeal.

The timing of the breakdown of resistance in this third trial concurs with that of the previous two studies where a rapid increase in FECs was observed in the last week of pregnancy and continued into lactation. The consistency of the timing of the breakdown throughout these studies, despite between-trial variation in LW change and CS of the animals, possibly highlights the lack of effect that these factors have in triggering the periparturient breakdown. The very marked synchrony in the timing of the breakdown with parturition has led other workers to suspect a link between the PPR and elevated plasma prolactin levels (Dunsmore, 1965; Salisbury and Arundel, 1970; Jeffcoate *et al.*, 1990). Interactions between the endocrine system, the periparturient breakdown and host nutrition require greater elucidation (Barger, 1993).

Immunologically induced suppression of faecal egg output generally relates to a reduction in the number adult worms or to a decrease in the fecundity of female worms (Michel, 1963; Dobson and Bawden, 1974). In the present study, differences in faecal egg output between P1 and P2 groups were clearly a reflection of the highly significant reduction in adult worm burden resulting from protein supplementation (Table 5.7). Protein supply had no effect on the fecundity of female worms (Table 5. 8). What was perhaps surprising however, was the significant effect of energy supply on in utero egg counts, but which was not reflected in the faecal egg outputs of E1 and E2 groups. The lack of effect on worm numbers may have masked the effect of fecundity differences.

There are several reports of arrestment or inhibition in the development of larvae as a result of acquired immunity (Michel, 1963; Urquhart *et al.*, 1966; Michel, 1974). The very low numbers of arrested larvae recovered in this trial (Table 5.7) and the failure to detect differences between treatment groups in the worm length, 21 days after cessation of infection (Table 5.8), suggest that inhibition and stunting were not a characteristic of the immune response in this study. The average lengths of worms observed in this study *viz.* 7.2 and 9.5 mm for male and female T. *circumcincta*, respectively, were consistent with the observations of Soulsby (1982) who reported *Teladorsagia* spp. lengths of 8.0 and 11.0 mm for male and female worms, respectively.

One can only speculate therefore, as to origin of the very significant differences observed in worm burdens. Resistance, manifested as low worm numbers, can result from a failure of incoming larvae to become established (Chiejina and Sewell, 1974b) or from the accelerated expulsion of adult stages (Jackson *et al.*, 1983). It is not possible, from the design of this study, to determine which of these two phenomena was involved - the three week gap between cessation of infection and slaughter being sufficiently long in duration to enable adults to reach patency and subsequent expulsion. However, the larval migration assay was included, in part, in an attempt to determine the importance of larval establishment in the subsequent worm burdens. It is believed that the antiparasitic qualities of mucus play a major part in the failure of incoming larvae to become established within the host gut (Lee and Ogilvie, 1981; Miller *et al.*, 1983; Kimambo and MacRae, 1988). The failure to detect differences in larval migration between nutritional treatment groups may indicate that inhibition of establishment was not a component of the enhanced resistance resulting from protein supplementation. The apparent correlation between T cell mitogens (Con A and PHA) and LMI trends may link leukotriene output with T cell responsiveness.

There are several reports indicating that the periparturient breakdown of resistance is species specific (Brunsdon, 1970; O'Sullivan and Donald, 1973; Gibbs and Barger, 1986). In the dual infection used in this study worm counts were significantly lower in *T. colubriformis* than *T. circumcincta* (Table 5.7). This might suggest that the relaxation of immunity to *Teladorsagia* spp. infection was greater than to *Trichostrongylus* spp. infection during the periparturient period, which is in agreement with the findings of Jackson *et al.* (1988) who reported that ewes in the latter stages of pregnancy expressed resistance to infection with *Trichostrongylus* spp. but were highly susceptible to infection with spp. What should be noted however, was that although worm burdens between species, were significantly different, the trends observed between treatment groups were similar for both species.

The lack of variation in lymphocyte response (SI) to mitogen and antigen stimulation between P1 and P2 groups suggests that the worm burden differences due to nutritional treatment in this trial are unlikely to be related to lymphocyte responsiveness. These results tend to contrast with the findings of van Houtert *et al.* (1995) who reported lymphocyte stimulation *in vitro*, in response to *T.colubriformis* third stage larval antigen to be significantly enhanced in lambs fed a diet supplemented with fishmeal. Other lymphocyte stimulation results in the van Houtert *et al.* (1995) study were however, equivocal with lymphocyte stimulation with PHA and LPS being unaffected by fishmeal supplementation while response to ovalbumin was lower in fishmeal supplemented lambs. These studies however, involved lambs and it is believed that immune factors are age dependent (Gregg *et al.* 1978; Kambara and McFarlane, 1996). The maintenance of resistance in older animals is considered to operate by additional mechanism which may well be temporarily suppressed by factors such as the endocrine changes which occur around parturition. Prolactin, has for many years been cited as involved in periparturient changes to host resistance to parasitic infection without conclusive proof (Coop *et al.*,

1990; Jeffcoate *et al.*, 1990). Alternatively the production of placental steroids and prostaglandins, known to increase markedly in late pregnancy and which are immunosuppressive may be involved. The responses to protein supplementation observed here may reflect the maintenance of resistance despite these endocrine changes or could indicate a more rapid re-establishment of resistance following breakdown, in late gestation.

Further study is required to determine precisely the role of nutrition in host resistance to parasitic infection. Whether the effect is due to protein supplementation per se. or to some other component of the fishmeal or to a specific amino acids remains unclear. Whatever the mechanism, it does not seem to be affecting antigen recognition and lymphocyte responsiveness. Parasitic infection is thought to increase amino acid requirements including the sulphur containing amino acids (Steel, 1978). It was suggested by McRae (1993) that amino acid demands were elevated as a result of parasitic infection and the associated local inflammatory response, including increased mucous secretion and leukotriene production. It may well be the case that the fishmeal provided in this study overcame a parasite induced deficiency of S-amino acids. It is plausible that partitioning of amino acids may alter in late pregnancy and lactation, directed more toward foetal growth and lactation at the expense of the immune system. This hypothesis is strengthened by the greater breakdown in resistance observed in twin bearing sheep. Clearly greater research attention is required in this area.

Chapter 6

The effect of fishmeal on parasite burdens of periparturient sheep

6.1 Introduction

Host resistance to parasitic infection may be manifested as an expulsion of incoming larvae, thus preventing establishment (Dobson *et al.*, 1990), a retardation of development of established larvae (Smith *et al.*, 1985; Jackson *et al.*, 1988), a reduction in the fecundity of female worms (Dobson and Bawden, 1974), and an expulsion of adult worms from the host gut (van Houtert *et al.*, 1995). The periparturient rise in FECs and worm burdens has been attributed to a temporary relaxation of these immune responses (Connan, 1968; O'Sullivan and Donald, 1970; Michel 1974,1976).

This chapter describes an experiment which aimed to expand on the previous results by determining the appropriate threshold level of protein supplementation required to minimise the extent of the periparturient breakdown. In addition, the study attempted to determine which of the immune mechanisms of resistance were affected by protein supplementation.

6.2 Materials and methods

Experimental Design

A group of thirty, mixed-age, female, Coopworth sheep, which had been oestrus synchronised, were identified as bearing twin lambs by ultra sound scanner (Aloka Echo camera, model SSD 210XII. Probe 3.5 MHz external, model UST-5021. Aloka Co. Ltd. Japan) ten weeks prior to parturition. The animals were assigned hierarchically according to LW and CS to three

nutritional groups (n=10), comprising three levels of dietary CP. Eight weeks prior to parturition they were drenched and housed in individual pens on slatted floors.

The sheep were trickle infected daily with 10,000 *T. circumcincta* and 7,000 *T. colubriformis* infective larvae during the 42 days before parturition. Infection stopped at parturition. Eleven days after parturition five animals were randomly selected from each group and given a single challenge infection of a further 25,000 *T. circumcincta* and 17,500 *T.colubriformis* infective larvae - thus two infection-regime groups were formed, *viz.* TO - trickle infection only and TC - trickle infection and challenge infection. All sheep were slaughtered ten days later .

Feeding and Management

Diets were designed to provide approximately the same level of ME to each group but to differ in their MP provision. This was achieved by the inclusion of 0, 100 and 200g of fishmeal kg^{-1} pellet concentrate in the diet of groups F0, F10 and F20, respectively. Regression equations derived from ME and MP requirement data of AFRC (1993) were used to calculate individual animal requirements based on LW as outlined in Appendix 5.1.1. Regression equations used in ration formulation in the present trial are given in Appendix 6.1. Initially the sheep were offered rations with ME levels designed to promote a maternal body weight loss of 50g day^{-1}. These requirements were met by feeding chaffed meadow hay and an appropriate pelleted concentrate in a ratio of 1:2 (Table 6.1). *In vitro* digestibility of organic matter of the feed was estimated using the cellulase/pepsin method of Jones and Hayward (1975). Metabolisable energy content of the forage was estimated using the formula of Barber *et al.* (1984) and for concentrates, the formula of Alderman (1985). Estimation of the level of ME provided by the diets was based on the methodology of AFRC (1993) which was summarised in Appendices 5.2.1 of

Chapter 5. The method of estimating MP content of the ration is outlined in Appendix 6.2. Calculation of undegradable (UP) and degradable protein (RP) content of feeds from weeks 14 to 17 of gestation was based on the mean of UP and RP values at rumen outflow rates 0.02 and 0.05 and described as UP 3.5 and RP 3.5. From week 18 until the end of the trial, rumen outflow rate was assumed to be 0.05 and UP and RP levels of feeds adjusted accordingly (Appendix 6.2). Based on these assumptions the diets were estimated to supply approximately 80, 100 and 125% of the predicted MP requirements (AFRC, 1993) to groups F0, F10 and F20, respectively. From five weeks prior to parturition feed levels in all groups were set to promote a maternal body weight loss of 100 g day^{-1}. During lactation a milk yield of 3.0 kg day^{-1} was assumed and all diets were designed to meet this requirement by providing 29.4 MJ ME day^{-1}, as recommended by AFRC (1993). Feed refusals were recorded daily. Dry matter percentage of refusals were assumed to be the same as DM of fresh feed offered. Estimated mean daily ME and MP intake levels of sheep in the three groups are shown in Table 6.2. Mean CP levels of the rations offered and estimated CP intake are given in Appendix 6.3.

Table 6.1 Composition and analysis of meadow hay & concentrate pellets offered to sheep during Trial 4 (g kg^{-1} DM)

	Feed			
	Pellet F0	Pellet F10	Pellet F20	Meadow Hay
Composition				
Chaffed lucerne hay	520	520	520	
Barley	430	330	230	
Molasses	50	50	50	
Fishmeal		100	200	
Analysis				
Dry matter	867	860	858	882
DOM[1]	851	817	826	619
Crude protein	185	259	336	168
M/D[2]	11.8	11.3	11.4	8.0
FME[3] (MJ kg^{-1} DM)	11.3	10.5	10.4	5.9
UP3.5[4]	24.0	58.0	90.0	6.0
RP3.5[4]	144.0	177.0	208.0	26.5
UP5[5]	28.0	66.0	103.0	7.0
RP5[5]	139.0	167.0	192.0	25.0

[1]DOM, digestible organic matter
[2]M/D MJ ME kg^{-1} DM
[3]FME Fermentable ME of diet (AFRC, 1993)
[4]UP3.5/ RP3.5 Undegradable/ degradable protein content at mean rumen digesta fractional outflow rate of 0.02 and 0.05/h. (AFRC, 1993)
[5]UP5/RP5 Undegradable/degradable protein content at mean rumen digesta fractional outflow rate of 0.05/h. (AFRC, 1993)

Table 6.2 Estimated mean daily metabolisable energy (MJ ME day^{-1}) and metabolisable protein (g MP day^{-1}) intake (offered - refused) (± SEM) of sheep around parturition in Trial 4

Week	F0		F10		F20	
	ME	MP	ME	MP	ME	MP
16	11.5 ± 0.33	84 ± 2.3	11.5 ± 0.31	109 ± 2.5	11.8 ± 0.22	135 ± 2.5
17	10.3 ± 0.34	75 ± 2.2	10.0 ± 0.60	94 ± 5.1	10.8 ± 0.21	121 ± 2.3
18	12.3 ± 0.37	94 ± 2.6	12.2 ± 0.28	124 ± 2.6	13.0 ± 0.24	160 ± 3.0
19	12.6 ± 0.41	96 ± 2.9	12.7 ± 0.34	127 ± 2.9	13.0 ± 0.24	160 ± 3.0
20	14.8 ± 0.44	118 ± 3.2	15.0 ± 0.53	155 ± 4.7	15.6 ± 0.25	197 ± 3.4
21	13.5 ± 0.36	111 ± 2.8	13.2 ± 0.46	146 ± 4.0	13.6 ± 0.27	186 ± 3.4
Lact. 1	24.4 ± 0.29	200 ± 2.0	23.7 ± 0.74	261 ± 7.2	23.9 ± 0.74	326 ± 10.2
Lact. 2	22.6 ± 1.41	185 ± 11.7	24.4 ± 0.35	268 ± 3.1	24.8 ± 0.29	338 ± 3.4
Lact. 3	23.0 ± 1.48	189 ± 12.1	24.1 ± 0.41	266 ± 4.4	24.5 ± 0.67	332 ± 8.9

General Methodology

Live weight was recorded weekly from housing and continued until the sheep were slaughtered, at the end of the trial, in the third week of lactation. Body condition score was assessed at fortnightly intervals from housing until two weeks prior to parturition as outlined in Chapter 3. A final assessment of CS was made immediately prior to slaughter. Lamb birth weight was recorded as soon as was practical after parturition. Lamb weight was also recorded at 'weaning' three weeks post partum.

Parasitology

At housing all animals were drenched with Ivomec (0.08 w:v Ivermectin, MSD AgVet, NZ) at a rate of 0.25 ml kg^{-1} LW. Infective larvae, dosing methods and parasitological techniques were similar to those described in Chapter 5. Faecal samples were collected at housing, to gauge initial parasite status, and then weekly from six weeks prior to parturition and until the animals were slaughtered in the third week of lactation. At slaughter abomasal and small intestinal worm burdens were determined as outlined in Chapter 5. Developmental stage, worm length and the number of eggs in the uteri of female worms was determined for both *Teladorsagia* and *Trichostrongylus* spp. using the method described for *Teladorsagia* spp. in Chapter 5.

Four, 12-month-old male Coopworth x Dorset Down sheep, which had been reared under conditions designed to prevent exposure to parasitic challenge, were dosed with 25,000 *T. circumcincta* and 17,500 *T.colubriformis* infective larvae, at the same time as the post partum challenge infection was administered to the TC ewes, using the same strain and batch of larvae. These four young sheep were slaughtered ten days later. The method of infection and the post slaughter procedure for worm burden determination were identical to those outlined above for ewes.

Computer tomography

Changes in bone, muscle and fat volume during the course of the experiment were estimated *in vivo* using X-ray computer tomography (CT). The principles of this method of prediction of body composition were summarised by Sorensen (1992). In the present study sheep were CT scanned in the week prior to housing *viz.* nine weeks before lambing and again, immediately prior to slaughter, three weeks after parturition. The sheep were fasted for a minimum of 12 hours prior to scanning and sedated with 1.0 ml 50 kg^{-1} LW of 10 mg ml^{-1} acepromazine ('ACEPRIL 10' Troy Laboratories Ltd, Auckland, NZ) intramuscularly, at least 30 minutes before scanning. They were further restrained as described by Nsoso (1995). Scanning was undertaken using the Cavalieri principle of Gunderson *et al.* (1988). In summary the animals were scanned at 18-20 equidistant sites along the long axis. The initial section was chosen at random in the neck region close to the head and subsequent sections were scanned at 55 mm intervals up to a point distal to the hind knee joint. Scanning procedure, data transfer and image analysis were as described by Nsoso (1995). Computer tomographic volumes for bone, muscle and fat were converted to weights by multiplying by standard density values of 1.549, 1.031 and 0.925 kg $dm^{3(-1)}$ for bone, muscle and fat, respectively (Nsoso, 1995).

Statistical analysis

Repeated measures analysis of variance (ANOVA) was undertaken on LW, CS and FECs using the general linear model procedure on the SYSTAT package (SYSTAT, 1990). Worm burdens, worm lengths, *in utero* egg counts, lamb birth weights and CT carcass composition change were analysed by ANOVA. Between group comparisons were made by calculation of least square differences used in protected tests only. Faecal egg counts and worm burdens were log transformed ($\log_{10}(\text{count} + 1)$) before analysis.

6.3 Results

Feed refusals increased in all groups with time. At parturition a number of sheep were found to have been incorrectly identified as bearing twin lambs. Actual pregnancy status is shown in Table 6.3. Pregnancy status of the animals was used as a covariate in analysis of LWT, lamb birth weight, CS, FECs and worm burden parameters as the results obtained in the previous work had indicated that pregnancy status had a significant effect on these factors.

Table 6.3 Pregnancy and suckling status of sheep in Trial 4

Group	Single	Twin	Triplet
F0	1	8	1
F10	2	3	5
F20	1	4	5

Live weight and body condition score

Live weight and CS during the experiment are shown in Figures 6.1 and 6.2. In the nine weeks prior to parturition, sheep in group F20 had a significantly greater LW gain than sheep in groups F0 and F10 - *viz.* 11.2 ± 0.63 kg for F20 sheep compared to 4.5 ± 0.58 kg and 6.8 ± 0.84 kg for F0 and F10, respectively ($P<0.01$). Live weight during lactation also changed significantly with time ($P<0.01$). This was strongly influenced by the number of lambs suckled *viz.* sheep suckling single lambs gained 1.0 ± 1.47 kg, while sheep suckling twins or triplets lost on average 1.2 ± 1.10 kg ($P<0.01$) (Figure 6.1). Nutritional treatment did not affect LW during lactation.

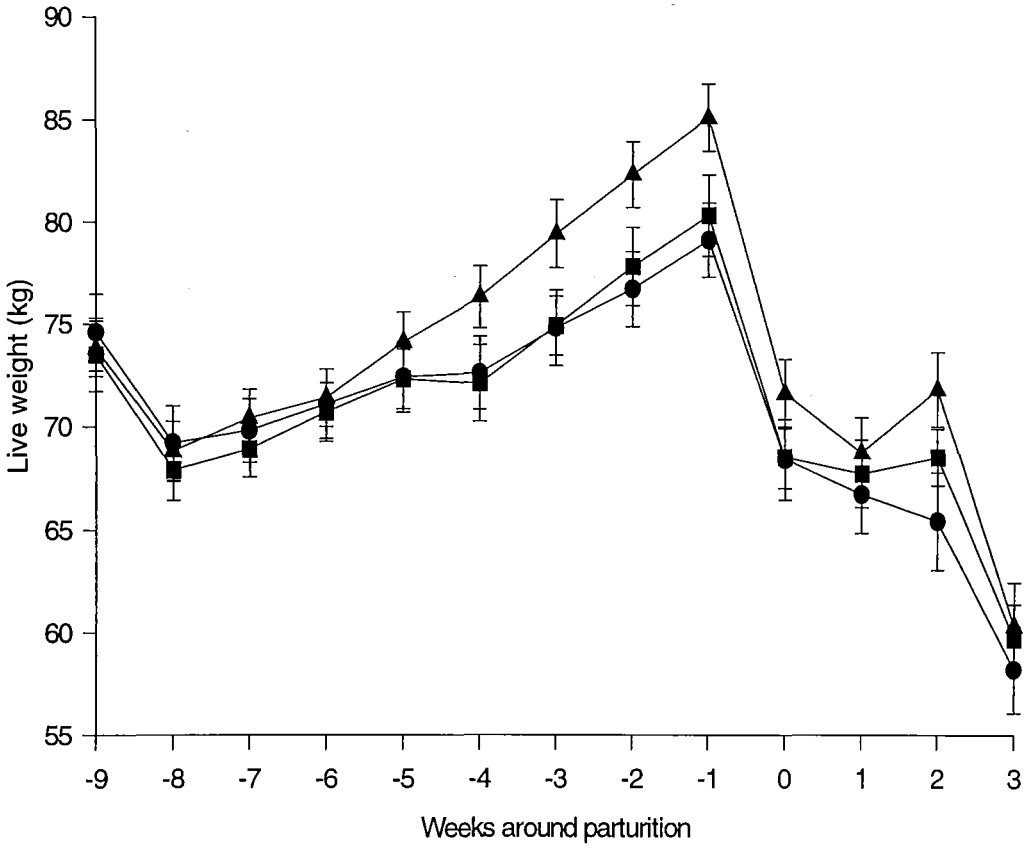

Figure 6.1 Mean live weight of sheep in groups FM0 (●) FM10 (■) and FM20 (▲) around parturition in Trial 4

Body condition score decreased in all groups prior to parturition ($P<0.01$) but there were no interactions between time and nutritional treatment or between time and pregnancy status. The CS determined immediately prior to slaughter indicated that sheep which had suckled a single lamb had a significantly higher CS than those which had suckled twins or triplets *viz.* 3.2, 2.0 and 1.7 CS for singles, twins and triplets, respectively ($P<0.05$).

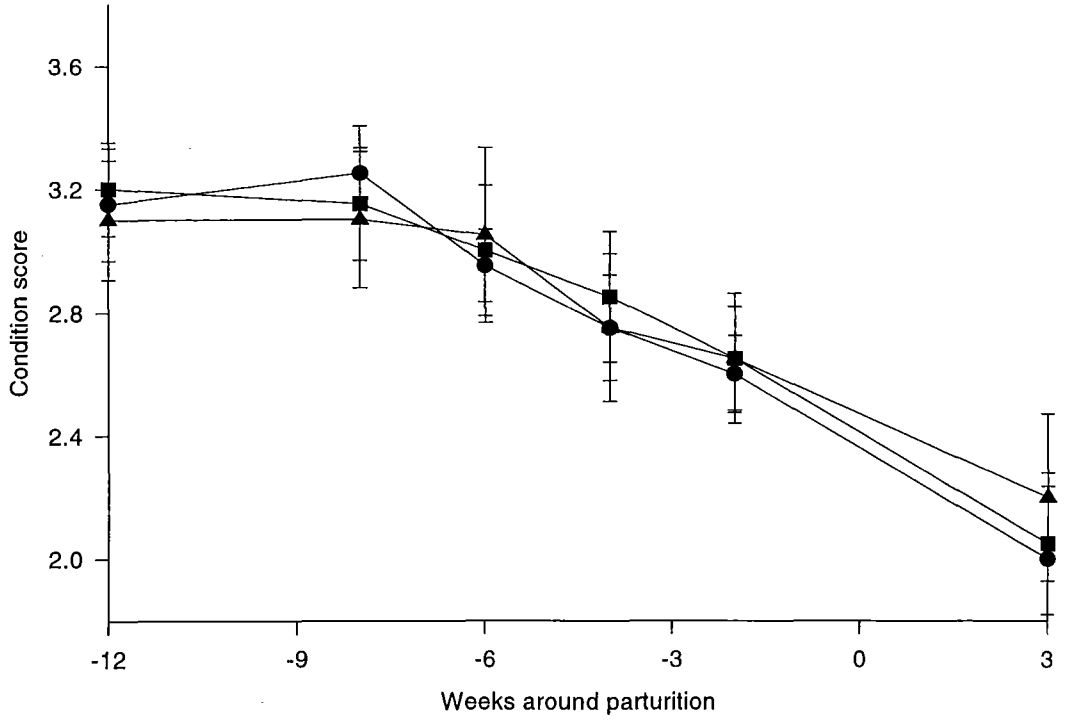

Figure 6.2 Mean condition score of sheep in groups FM0 (●) FM10 (■) and FM20 (▲) around parturition in Trial 4

Lamb birth weight and weaning weight three weeks after parturition and adjusted for birth status are shown in Table 6.4. Both were significantly affected by the presence of siblings ($P < 0.01$) but there was no significant effect of protein supply on birth weight or weaning weight.

Table 6.4 Mean individual lamb birth weights, weaning weights (21 days post partum) and daily growth rate of lambs (all adjusted for birth status) in Trial 4

Group	Mean birth weight (kg)	Mean weaning weight (kg)	Growth rate (g day^{-1})
F0	4.4 ± 0.19	8.7 ± 0.50	205
F10	4.3 ± 0.15	8.8 ± 0.59	214
F20	4.7 ± 0.13	9.9 ± 0.46	248

nb. No significant differences ($P>0.05$)

Computer tomographic estimates of body composition

Actual carcass weights of the sheep at the end of the trial were compared with CT estimated carcass weight (Table 6.5). There was significant variation between the two weights although the r value 0.919 suggested that this was a consistent discrepancy. Computer tomographic estimations of bone, muscle and fat weight were adjusted to the actual carcass weight (Appendix 6.4). Adjusted estimates of carcass weights at nine weeks prior to parturition and three weeks afterwards are shown in Table 6.6. Changes in estimated CT bone, muscle and fat weights are shown in Table 6.7. Estimated bone weight decreased in all groups with the greatest decrease of 0.4 ± 0.10 kg observed in F0 sheep and the least, 0.3 ± 0.17 kg observed in F20 sheep. Groups F0 and F10 lost an estimated 1.4 ± 0.46 and 0.1 ± 0.51 kg of muscle tissue, respectively, while sheep in Group F20 gained an estimated 0.4 ± 0.92 kg. Estimated fat weight decreased by 3.0 ± 0.45, 3.3 ± 0.62 and 3.2 ± 1.1 kg in groups F0, F10 and F20, respectively. There was no significant effect of nutritional treatment on these changes in CT estimated body composition. Single bearing and suckling ewes gained on average 0.7 ± 0.92 kg of fat tissue as estimated by CT. This was significantly different from the estimated fat loss observed in the twin and triplet bearing ewes which was estimated to be -3.2 ± 0.40 kg and -4.6 ± 0.58 kg, respectively ($P < 0.01$). The CT estimated gain in fat tissue observed in single bearing/rearing sheep resulted in the overall change in CT carcass weight of these animals which was significantly less than that of twin and triplet bearing/rearing ewes *viz.* an overall reduction in estimated carcass weight of 0.1 ± 0.45 kg in single, compared with 3.8 ± 1.02 kg and 5.4 ± 1.30 for twin and triplet bearing ewes, respectively.

Table 6.5 Pre-adjusted computer tomography (CT) estimated mean carcass weight (CT Bone + CT Muscle + CT Fat) (± SEM) nine weeks prior to parturition (T1) and three weeks post partum (T2) and actual mean carcass weight at slaughter

Group	CT estimated carcass weight		Actual carcass weight
	T1 (kg)	T2 (kg)	T2 (kg)
F0	35.6 ± 1.42	29.3 ± 1.31	22.5 ± 1.29
F10	35.3 ± 1.17	30.4 ± 1.49	24.0 ± 1.35
F20	35.2 ± 1.45	31.4 ± 1.80	24.0 ± 1.44

nb. No significant differences (P>0.05)

Table 6.6 Computer tomography (CT) estimated mean carcass weight (CT Bone + CT Muscle + CT Fat) (± SEM) nine weeks prior to parturition (T1) and three weeks post partum (T2) adjusted for actual carcass weight

Group	T1 (kg)	T2 (kg)
F0	27.3 ± 1.40	22.5 ± 1.29
F10	27.8 ± 1.00	24.0 ± 1.35
F20	27.1 ± 1.58	24.0 ± 1.44

nb. No significant differences (P>0.05)

Table 6.7 Computer tomography (CT) estimated bone, muscle and fat weight (± SEM) of sheep nine weeks prior to parturition (T1) and three weeks post partum (T2) adjusted for actual carcass weight

	Bone		Muscle		Fat	
Grp	T1 (kg)	T2 (kg)	T1 (kg)	T2 (kg)	T1 (kg)	T2 (kg)
F0	2.9 ± 0.15	2.5 ± 0.09	13.6 ± 0.77	12.2 ± 0.60*	10.9 ± 0.84	7.9 ± 0.79
F10	3.2 ± 0.11	2.8 ± 0.09	13.3 ± 0.54	13.2 ± 0.47	11.4 ± 0.78	8.1 ± 1.30
F20	3.1 ± 0.19	2.8 ± 0.29	13.7 ± 0.97	14.1 ± 0.39*	10.3 ± 0.69	7.1 ± 1.14

*Means marked thus differ statistically from each other ($P<0.05$)

Parasitology

The initial faecal sample obtained at housing, eight weeks before lambing, identified only three individuals with positive FECs, all at the minimum detectable level of 100 epg. Subsequent FECs are shown in Figure 6.3. During the 6 weeks before lambing egg counts increased significantly with time ($P<0.01$) but there was no time by treatment interaction or time by pregnancy status interaction. Mean counts remained below 100 epg in all three groups prior to parturition. Counts increased rapidly post-partum in groups F0 and F10, and exceeded 600 epg in both groups by the third week of lactation. Faecal egg counts of group F20 remained below 100 epg throughout the course of the experiment; however group differences were significant only on days seven and seventeen post partum ($P< 0.05$).

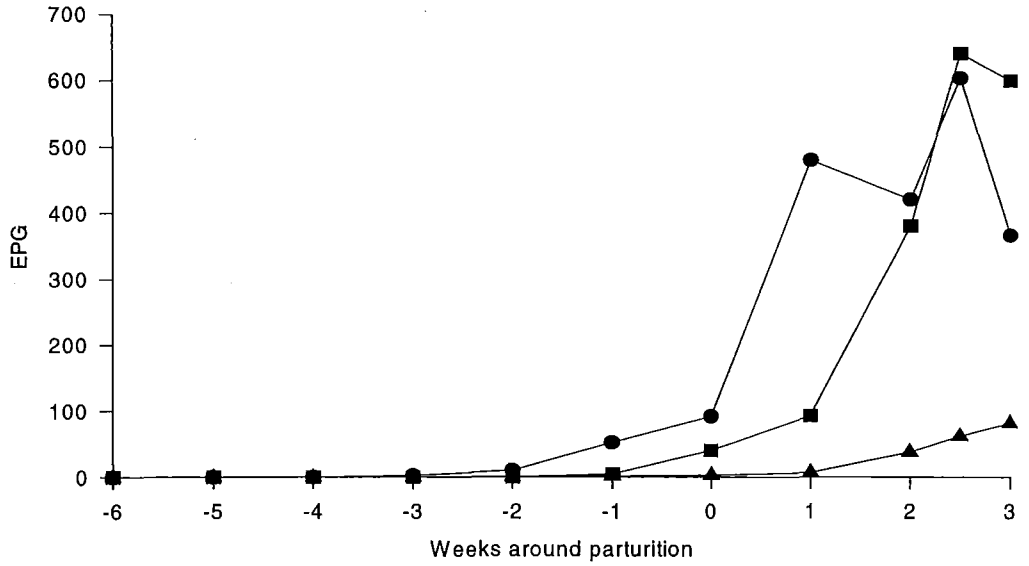

Figure 6.3 Geometric mean (log_{10}(count+1)) faecal egg count (EPG) of sheep in groups F0 (●), F10 (■) and F20 (▲) around parturition in Trial 4

Faecal egg counts of T0 and TC groups following the post partum challenge infection are shown in Figures 6.4.1. Sheep which received both a trickle and the post partum challenge infection (TC) had significantly lower FECs immediately prior to slaughter than sheep which had received only the trickle infection (TO) ($P< 0.05$). This trend was evident in all three nutritional groups as shown in Table 6.8 and Figures 6.4.2-6.4.4.

Table 6.8 Comparison of final three geometric mean (Log_{10} (count+1)) faecal egg countsof sheep in groups TO (trickle infection only) and TC (trickle infection and post partum challenge infection)

Group	F0		F10		F20	
Days post infection	TO	TC	TO	TC	TO	TC
3	252	704	793	183	42	34
6	505	723	1047	392	377	9*
10	620	217	1048	344	625	10

* This mean value differs significantly from other TC means in this row ($P<0.05$). All other means no significant difference ($P>0.05$)

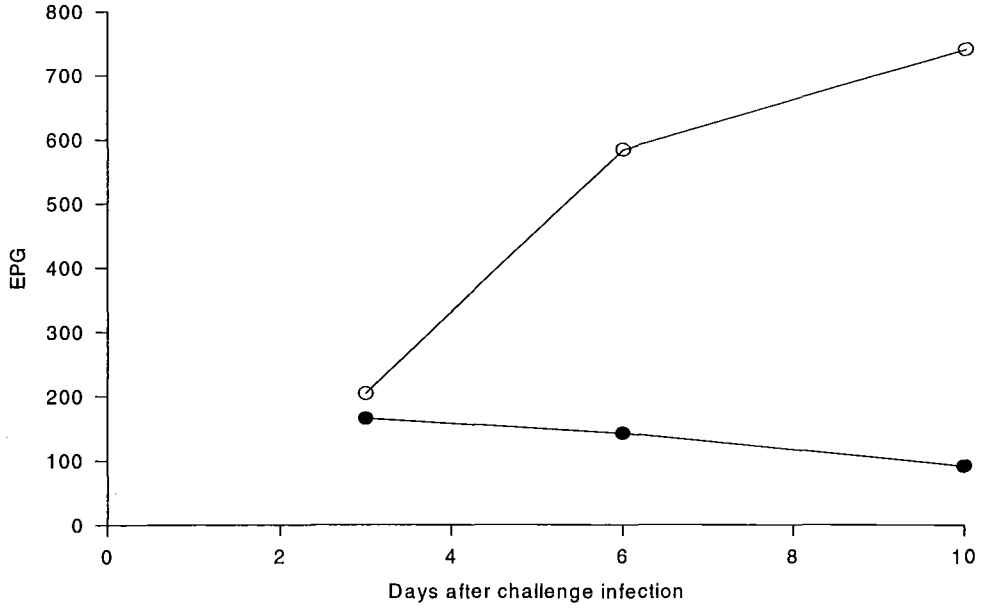

Figure 6.4.1 Comparison of geometric mean (log_{10}(count+1)) faecal egg count (EPG) between sheep trickle infected only (TO - /) and sheep trickle infected and post partum challenge infected (TC - ●) in Trial 4 (nb. Pooled FECs of all groups)

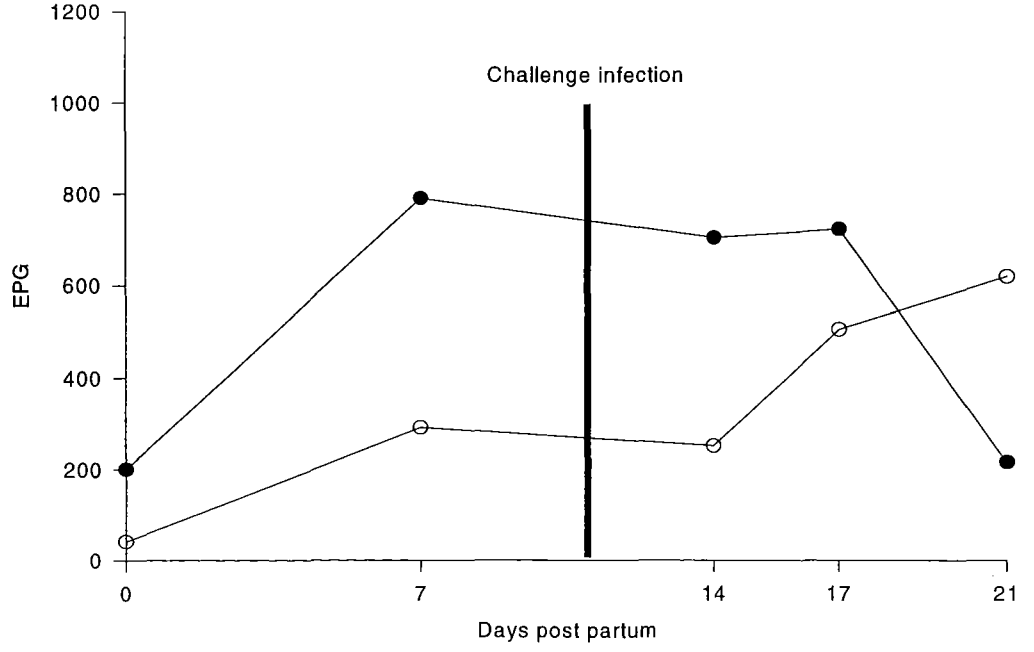

Figure 6.4.2 Geometric mean (log_{10}(count+1)) faecal egg count (EPG) of sheep in group FM0 trickle infected only (TO - /) and sheep trickle infected and post partum challenge infected (TC - ●)

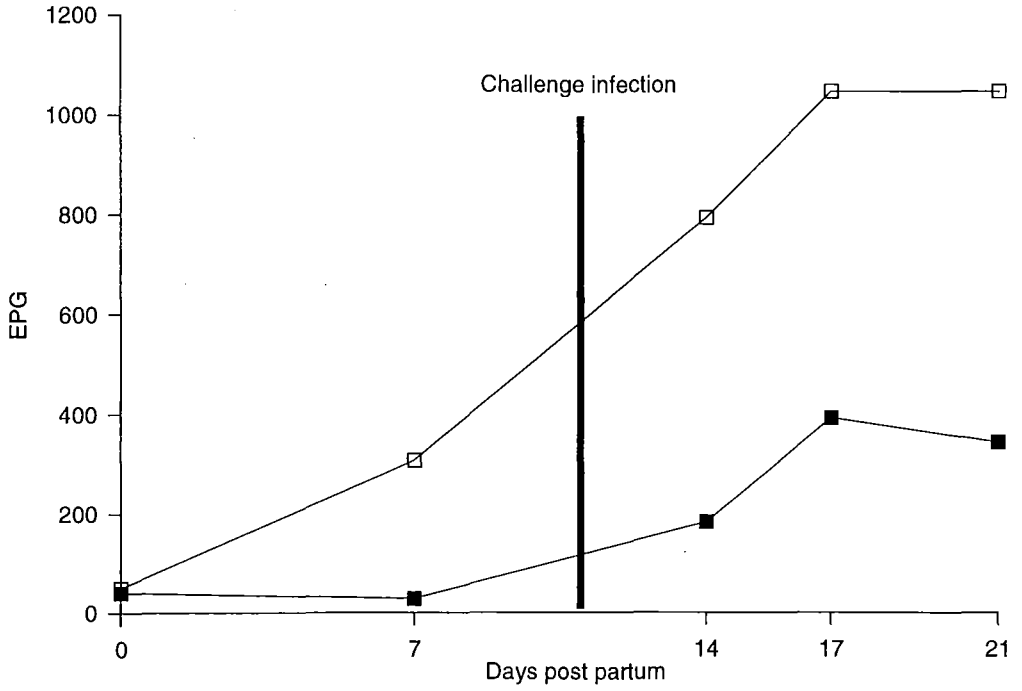

Figure 6.4.3 Geometric mean (log_{10}(count+1)) faecal egg count (EPG) of sheep in group FM10 trickle infected only (TO - ❑) and sheep trickle infected and post partum challenge infected (TC - ■)

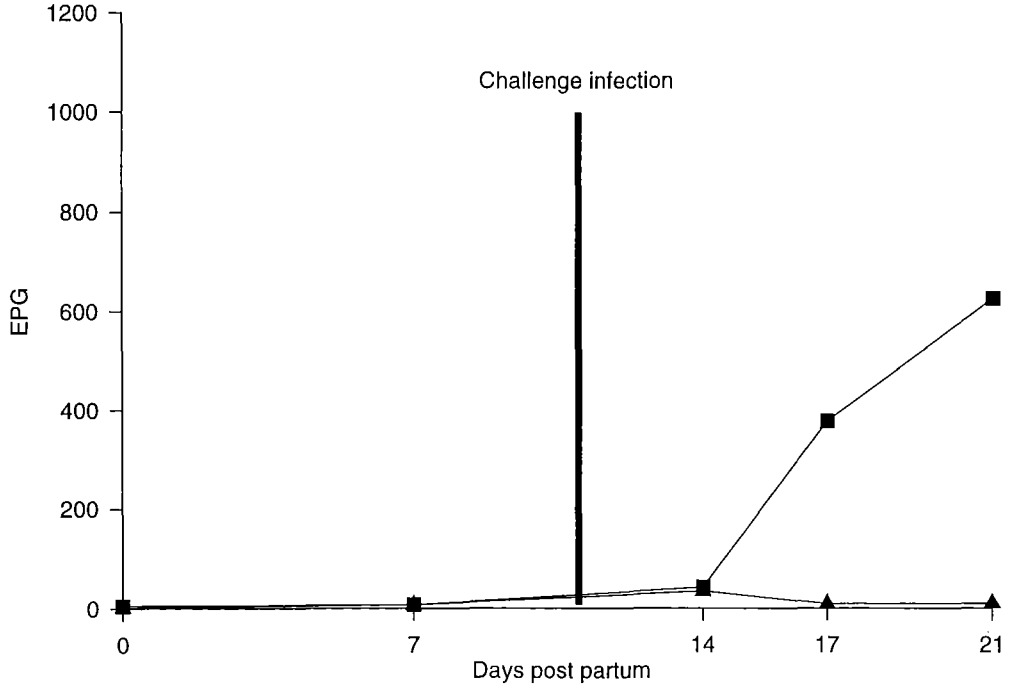

Figure 6.4.4 Geometric mean (log10(count+1)) faecal egg count (EPG) of sheep in group FM20 trickle infected only (TO-■) and sheep trickle infected and post partum challenge infected (TC -▲)

Geometric mean *Teladorsagia* and *Trichostrongylus* spp. burdens are shown in Tables 6.9 and 6.10, respectively. Worm burdens of both larval stages and of both species decreased with increasing protein supply. F20 sheep had significantly fewer L4 and L5 worms of both species than F0 sheep ($P<0.05$). Similarly F10 sheep had lower burdens than F0 sheep but these differences were not statistically significant. There were significantly fewer L5 *Teladorsagia* spp. recovered from sheep in group F20 than from sheep in group F10 ($P<0.05$).

Sheep which received the trickle infection only (TO) had significantly lower L4 burdens than the challenged TC sub-groups ($P< 0.01$). The difference in L5 burdens between TO and the TC sub-groups was not significant. However, there was a trend toward lower L5 worm burdens in sheep challenged post partum ($P=0.09$), with the exception of *Trichostrongylus* spp. burdens in sheep on the F0 nutritional treatment. There were no L4 larvae recovered from the digested abomasa of TO animals.

Percentage establishment of the post partum challenge infection was calculated to be 15.2, 9.4 and 5.9% in *Teladorsagia* spp. and 9.3, 3.4, and 0.9% in *Trichostrongylus* spp. for groups F0, F10 and F20, respectively. Differences in establishment were significant in *Trichostrongylus* spp. only and only between sheep in groups F0 and F20 ($P<0.05$). Establishment rates were significantly lower for *Trichostrongylus* spp. than for *Teladorsagia* spp. ($P<0.05$). Establishment rates of the four young sheep are given in Table 6.11. The average rate of establishment of *T. colubriformis* was 31.5% (range 28 - 40%) and *T. circumcincta* was 33.5% (range 2 - 52%).

There was a tendency toward increased L5 *Teladorsagia* spp. burden with increasing ewe fecundity in TC groups, *viz.* 1430, 2775 and 5355 for single, twin and triplet bearing sheep, respectively ($P=0.06$) and significantly higher L5 *Trichostrongylus* spp. burdens with increasing fecundity in TC groups - *viz.* 0,

220 and 2050 in single, twin and triplet bearing sheep, respectively ($P<0.05$). There was no effect of ewe fecundity on the worm burdens of TO sheep. Pregnancy status had no effect on the other worm burden parameters measured *viz.* ratio of male to female worms, worm length and *in utero* egg counts.

The mean ratio of male to female L4 and L5 worms was 1:1.6 in both *Teladorsagia* and *Trichostrongylus* spp. (Tables 6.9 and 6.10). Ratios were not assessed between male and female L4 larvae from TO sub-groups because of the very low numbers present. There were no significant differences in the ratio of male to female worms resulting from nutritional treatment.

Table 6.9 Geometric mean (Log10 (count + 1)) *Teladorsagia* spp. worm burdens (range) of sheep three weeks post partum and ratio of male to female L5 in groups TO (trickle infection only n = 5) and TC (trickle infection + post partum challenge n = 5)

Group	L4		L5		Ratio L5 F:L5 M	
	TO	TC	TO	TC	TO	TC
F0	1[a] (0 – 330)	3400[a] (1430 – 6600)	10040[a] (4427 – 13678)	6490[a] (4589 – 8000)	1: 2.2[a]	1: 1.1[a]
F10	2[a] (0 – 110)	1940[a] (660 – 4180)	6400[a] (3160 – 13274)	4295[ab] (1428 – 10704)	1: 1.7[a]	1: 1.8[a]
F20	0[a] (0)	1340[a] (770 - 2310)	2780[a] (721 - 9751)	1295[a] (120 - 5394)	1: 1.8[a]	1: 1.2[a]

Means with different superscripts within columns indicates significant difference (P<0.05)

Table 6.10 Geometric mean (Log10 (count + 1)) *Trichostrongylus* spp. worm burdens of sheep (range) three weeks post partum and ratio of male to female L5 in groups TO (trickle infection only n = 5) and TC (trickle infection + post partum challenge n = 5)

Group	L4		L5		Ratio L5 F:L5 M	
	TO	TC	TO	TC	TO	TC
F0	1[a] (0 – 110)	1200[a] (220 – 3300)	460[a] (110 – 8910)	9650[a] (660 – 49610)	1: 1.5[a]	1: 1.7[a]
F10	7[a] (0 – 110)	150[ab] (0 – 1650)	780[a] (0 – 43560)	230[ab] (0 – 15950)	1: 1.6[a]	1: 1.5[a]
F20	0[a] (0)	8[b] (0 - 550)	110[a] (0 - 9350)	14[b] (0 - 8250)	1: 2.0[a]	1: 1.1[a]

Means with different superscripts within columns indicates significant difference (P<0.05)

Table 6.11 Worm burdens recovered from previously naive young sheep challenge infected with 17,500 *T.colubriformis* and 25,000 *T. circumcincta* and slaughtered ten days later

ID	*T.colubriformis*	% Established	*T. circumcincta*	% Established
136	5170	29.5	556	2.2
137	7040	40.2	9308	37.2
138	4950	28.3	13000	52.0
139	4840	27.6	10700	42.8

Teladorsagia and *Trichostrongylus* spp. worm lengths and *in utero* egg counts are shown in Tables 6.12 and 6.13, respectively. Due to the low numbers of *Trichostrongylus* spp. recovered, measurement of worm length and *in utero* egg counts for this species were based on samples from fifteen sheep randomly selected but balanced for numbers between TO and TC groups. *Teladorsagia* spp. adults had a mean length of 7.2 and 9.5 mm and adult *Trichostrongylus* spp. a mean of 4.2 and 4.9 mm respectively, for male and female. There was no effect of nutritional treatment, pregnancy status or post partum challenge infection on worm length.

Teladorsagia spp. had a mean of 33 eggs *in utero* and *Trichostrongylus* spp. a mean of 11. *Teladorsagia* spp. worms recovered from animals challenged post partum (TC groups) had significantly fewer eggs *in utero* than those in TO groups - *viz.* 26 and 39 eggs, respectively ($P < 0.01$). There was no effect of nutritional treatment on *in utero* egg counts.

Table 6.12 Mean *Teladorsagia* worm lengths (± SEM) and in utero egg counts (±SEM)

Group	Worm length (mm)						Eggs *in utero*	
	TO			TC			TO	TC
	n	Female	Male	n	Female	Male		
F0	5	9.7 ± 0.03	7.5 ± 0.01	5	9.3 ± 0.02	6.9 ± 0.02	40 ± 4.9	29 ± 2.6
F10	5	9.8 ± 0.03	7.4 ± 0.02	5	9.4 ± 0.03	7.2 ± 0.02	39 ± 2.5	28 ± 4.6
F20	5	9.6 ± 0.03	7.1 ± 0.02	4	9.0 ± 0.05	7.0 ± 0.03	37 ± 5.1	22 ± 4.3

Table 6.13 Mean *Trichostrongylus* worm lengths (± SEM) and in utero egg counts (± SEM)

Group	Worm length (mm)						Eggs *in utero*	
	TO			TC			TO	TC
	n	Female	Male	n	Female	Male		
F0	1	4.3 ± 0.00	4.3 ± 0.00	4	5.2 ± 0.01	4.3 ± 0.02	11 ± 0.0	10 ± 1.9
F10	3	5.0 ± 0.01	4.2 ± 0.02	2	5.0 ± 0.02	4.2 ± 0.02	11 ± 0.3	10 ± 0.5
F20	3	5.0 ± 0.01	4.0 ± 0.01	2	4.6 ± 0.01	3.9 ± 0.01	13 ± 0.7	14 ± 1.5

6.4 Discussion

The results presented here confirm those of Chapter 5 that the periparturient breakdown in resistance can be moderated by nutritional supplementation. The effect appeared to follow a linear relationship - the evidence for resistance being greater with an increasing level of supplementation. The trend for occurrence of greater resistance in less fecund sheep confirms the findings of Chapter 5 and possibly add support to a nutritional status susceptibility. In addition, this trial provides evidence that the effects on worm burdens may be occurring at the establishment stage, against incoming larvae.

Relative changes to body composition during the course of the experimental period were demonstrated by CT estimates. The estimates depend on precise counts of pixel frequency within areas of tissue which vary in their specific density on a grey scale, *viz.* bone is more dense than muscle tissue which is more dense than fat tissue. Pixel frequency in each specified grey scale range is subsequently converted to estimated tissue volume using standard density conversion factors. The precise cause of the discrepancy between actual carcass weight and CT estimated carcass weight could not be determined. Linear dimensions on a scanned object (scanning box) were found to be almost identical when measured within an image, providing good evidence that actual volume and CT estimated volume were similar. This does not rule out errors arising in segregation of pixels on the basis of grey scale associated with the three body tissues. The ability to accurately identify fat, muscle and bone from a range of grey scales is likely to have a significant effect on final tissue weights. Alternatively the discrepancy between CT estimated and actual carcass weight may have been a result of the removal of pelage, skin, hock, and upper neck from the carcass which was present in the image at the time of CT analysis, thus giving an over estimated CT carcass weight. As the variation was consistent across all animals and it was the relative changes in bone, muscle and

fat weight which were of interest, rather than the absolute values, it was considered justifiable to adjust CT weights based on actual carcass weight.

The results obtained from CT estimation of body composition changes resulting from the different levels of protein supply tend to agree with the observations of Cowan *et al.* (1979, 1980) that protein supplementation is likely to influence body fat reserves but will have very little effect on body protein. It is believed that increased protein supply is likely to increase the availability of essential amino acids for milk production. The increase in milk production increases energy requirement hence the utilisation of body fat reserves. That protein supply was, in part, indirectly responsible for increased energy demand and therefore, the loss of fat tissue, is further implied by the increased rate of growth in lambs born to F20 ewes *viz.* 248 g day^{-1} compared with the growth rates of 205 and 214 g day^{-1} for lambs born to F0 and F10 ewes, respectively. These results contrast with the reports of Robinson and Forbes (1968) who fed ewes in the first three weeks of lactation, rations, varying in their CP content, and observed lamb growth rates of 310 and 230 g day^{-1} for CP intakes of 61 and 157 g day^{-1}, respectively. Unfortunately the final measurement of ewe LW in the present study was made after the period of pre-slaughter fasting and as a result the information on mean ewe weight change during lactation is of limited value. Robinson and Forbes (1968) found that their ewes lost 190 g day^{-1} on the low CP ration and gained 10 g day^{-1} on the higher level.

The parasitological findings clearly indicated that adult worm burdens decreased significantly with increasing dietary protein supply. Furthermore the fact that the TC sub group harboured two distinct worm populations - *viz.* an immature L4 burden as well as an adult worm burden, while the TO animals harboured only an adult population, clearly suggests that L4 larvae present in the TC sheep had originated almost entirely from the post partum challenge. Moreover, since larval (L4) establishment in the TC groups was also affected by

protein supply it seems plausible to conclude that protein supply affected events which limit larval development in the period 1-10 days after infection.

The absence of inhibited larvae in T0 groups, regardless of protein supplementation, is perhaps surprising and may point to a periparturient relaxation of resistance having occurred across all three treatment groups. There are many reports in the literature of the periparturient breakdown being closely associated with resumption of development of previously arrested larvae (Connan 1968; O'Sullivan and Donald 1970; Michel 1974; Gibbs and Barger 1986). This would tend to suggest that the response to protein supplementation observed in the present study *viz.* significantly lower worm burdens in F20 sheep, may have resulted from the effects of fishmeal on the re-establishment of - rather than on the maintenance of resistance. With hindsight, a serial slaughter of ewes may have provided more information on the levels of inhibition occurring at different stages of the infection period in late pregnancy. Additionally, one cannot discount the possibility that a very low inhibition rate may have been a characteristic of this particular strain of *T.circumcincta.*

Much of the research on the interaction between host nutrition and resistance to infection has been undertaken in young growing animals where immune mechanisms of resistance may differ from those of the mature sheep used in the present work. Several workers have found that protein supplementation appears to enable the young animal to withstand the pathophysiological consequences of infection and to expel established adult worms rather than prevent establishment of infection (Bawden, 1969; Dobson and Bawden, 1974; Abbott *et al.*, 1985a). Many of these studies however, involved large single infections unlike the prolonged trickle infection of the present work. Establishment rates may have been affected as trickle infection progressed.

In a study by Bown *et al.* (1991), 3.5 month old sheep undergoing challenge infection of 3,000 *T.colubriformis* larvae day^{-1} were abomasally infused with

isocaloric amounts of either protein or energy. Despite a significant difference in nitrogen intake between the protein and energy groups there was no difference in FECs or worm burdens of sheep slaughtered after 6 weeks of infection but by 12 weeks, FECs and worm burdens of sheep supplemented with protein were significantly reduced. This may indicate that establishment rates and adult worm survival were unaffected by nutrient plane during the early stages of infection but in the latter stages protein supplemented sheep were able to mount some form of immune response which was manifested as the reduction in FECs and worm burdens.

van Houtert *et al.* (1995) reported a similar trend where the effect of fishmeal supplementation was monitored in young lambs trickle infected with *T.colubriformis* and slaughtered at either 35, 70, 105 or 140 days of infection. Protein supplementation had no effect on worm burdens of lambs slaughtered after 35 or 70 days of infection, but significant reductions were observed after 105 and 140 days of infection. In this study, trickle infection ceased 16 days prior to slaughter and all animals were given a further single infection nine days before being slaughtered to determine larval establishment rates. Establishment rates were not affected by protein supplementation at any time, suggesting that worm burden differences achieved in the prolonged infections had occurred, in this case, due to expulsion of already established worms and not by the development of resistance to incoming larvae. In the study of Bown *et al.* (1991) the reductions in FECs and worm burdens observed after 12 weeks of infection may also have reflected the enhanced expulsion of established larvae.

In the present study, larval establishment rates were determined only for sheep given the challenge infection post partum. Although there was a trend toward decreasing establishment with increased protein supply, establishment was significantly different only with *Trichostrongylus* spp. between groups F0 and F20. One could speculate that the rate of establishment may have varied

throughout the trial period, however without serial slaughter to provide information on worm burdens during the course of the trickle infection period this cannot be substantiated. Establishment rates of the four naive sheep were relatively high in comparison to the main trial ewes and this could indicate that the degree of breakdown of resistance in the adult sheep may have been relatively limited in extent. The highest rate of establishment of *Trichostrongylus* spp. - 9.3% in F0 ewes, was only 30% of the establishment of *Trichostrongylus* spp. in naive lambs.

The results obtained in the present study tend to suggest that responses to protein supplementation in the mature animal may differ from those of young stock. Resistance in the protein supplemented groups appeared to reflect, in part at least, an effect on the animal's ability to reject incoming larvae, thus overcoming the loss of this particular regulatory mechanism as highlighted by O'Sullivan and Donald (1970, 1973). However, this need not necessarily have been the only immune mechanism responsible for the suppressed worm burdens in protein supplemented sheep.

Clearly the precise role of protein supplementation in the immune response requires more detailed analysis. Sheep appear to have little innate immunity to parasitism and are often several months old before they acquire resistance to helminth infection (Manton *et al.*, 1962; Ross, 1970; Gibson and Parfitt, 1972; Nansen, 1985). The protein supplementation studies described above (Bawden, 1969; Dobson and Bawden, 1974; Abbott *et al.* 1985a, Bown *et al.*, 1991; van Houtert *et al.*, 1995), which failed to show an effect on the rate of parasite establishment, may well have demonstrated the lack of effect of nutrition on enhancing this innate immunity. In addition to this, rejection of incoming larvae may not be a component of the immune response in sheep until a threshold level of infection is experienced (Dineen, 1963; Chiejina and Sewell, 1974a) - perhaps occurring after 10 - 12 weeks of infection. Immune responses and the regulatory mechanisms through which these operate remain equivocal

and require further investigation. Whether nutrition affects the host's ability to act in response to antigenic stimulation or simply enables the host to recognise infection remains unknown.

The mean lengths of worms recovered in this experiment *viz.* 7.2 and 9.5 mm for male and female *Teladorsagia* spp., respectively and 4.2 and 4.9 mm for male and female *Trichostrongylus* spp., respectively, fit closely with the descriptions of Soulsby (1982) who estimated *Teladorsagia* spp. at 8.0 and 11 mm, respectively for male and female and *Trichostrongylus* spp. as 4.7 and 6.0 mm, for male and female, respectively. Although worm stunting is considered a separate immune phenomena to inhibition the two often occur together (Michel, 1963). Yet, as the present results indicate, worm length did not appear to be affected by protein supplementation. The lack of worm stunting may again reflect a periparturient breakdown of resistance having occurred in all three nutritional groups. Alternatively though, worm stunting may simply not have been a factor in the immune response in these animals.

The suppression of FECs below 100 epg in the F20 groups clearly indicates a trend toward reduced FECs with increased supply of fishmeal though statistical significance was not achieved throughout. This was perhaps surprising since in the previous experiment (Chapter 5) had demonstrated consistent and significant differences in FECs of sheep offered diets which varied by only 20g day^{-1} in estimated MP supply, considerably less than the difference between F0 and F20 in the present study. In both cases the breakdown of resistance, as manifested by a rise in egg count, occurred in the first week after parturition. In the present study egg counts averaged only 425, 350 and 36 epg for sheep in groups F0, F10 and F20, respectively, in the first three weeks of lactation, while in the earlier study (Chapter 5) egg counts during lactation averaged 890 epg for low protein sheep and 150 epg for high protein sheep. This may serve to highlight the variability which can occur in FECs and casts doubt on their usefulness as sole indicators of parasite disease status. It may also question the

value of comparing parasite trials between years as a number of factors, including larval intake (pre-housing), which may well have varied between years, are likely to have affected the results obtained.

Acquired immunity to nematodes may also be manifested as a reduction in the fecundity of female worms (O'Sullivan and Donald, 1970; Dobson and Bawden, 1974). In the present work however, *in utero* egg counts did not vary significantly between nutritional treatment groups (Tables 6.12 and 6.13). Again, the lack of effect of nutritional treatment on this immune response tends to indicate that resistance in all sheep in the periparturient period had been equally compromised, regardless of protein supplementation. Interestingly, the average *T.circumcincta in utero* egg count across all T0 sheep, *viz.* 38.6, was of similar magnitude to that reported by McAnulty (1990) in ewes given a single challenge dose of 20,000 *Teladorsagia* spp. either at lambing or six weeks after lambing. During these periods McAnulty's (1990) ewes were clearly susceptible to infection and were reported to have *in utero* egg counts of 45 and 38, for partum and six week post lambing infection, respectively. The lack of effect on worm fecundity in the present work may also indicate that this particular component of the immune response was not sensitive to protein supplementation or that it may be the last to re-establish after the periparturient relaxation. This result, together with the lack of difference between the ratios of male to female worms would suggest that differences in FECs were related purely to worm numbers rather than some other immune factor.

More detailed analysis of the FECs indicated that sheep, challenge infected post partum, had significantly lower egg counts than those trickle infected only (Table 6.8) *viz.*- 220, 340 and 10 epg for TC sheep, compared with 620, 1050 and 620 epg for TO sheep in groups F0, F10 and F20, respectively ($P < 0.05$). This finding was accounted for by the significantly lower numbers of nematode eggs in the uteri of worms recovered from animals in TC groups than in TO groups ($P < 0.01$). This could indicate that the challenge larval dose,

administered eleven days after the cessation of the trickle infection, may have reduced the fecundity of the worms through some worm population interaction similar to that observed by Chiejina and Sewell (1974a and 1974b). These workers suggested that inhibition of ovulation in worms may not be caused entirely by immunological mechanisms but may also be affected by the numbers of worms present. The reduction in FECs resulting from the post partum challenge infection may partly account for the trend toward lower egg counts observed in this trial than previously, in Trial 3 (Chapter 5).

The post partum challenge infection also appeared to reduce adult worm numbers which would clearly add to the reduction in FECs. The occurrence of an abrupt loss of worms resulting from a challenge infection - termed 'self-cure`- is thought to be a hypersensitive reaction induced by previous, prolonged exposure to larvae and has been observed in *Haemonchus*, *Trichostrongylus* and *Teladorsagia* spp. infections (Stewart, 1953; Hong *et al.*, 1989).

It cannot be concluded that the differences in worm burdens observed between nutritional treatment groups were related solely to a variation in the rate of establishment of incoming larvae. The fact that adult worm burdens were apparently sensitive to the post partum challenge infection *viz.* adult worm burdens were lower in sheep infected post partum, could be used to develop the argument that the differences in worm burdens reported in Tables 6.9 and 6.10 may also have resulted from expulsion of existing adult worms.

In summary, the results of the present study may indicate that protein supplementation enabled sheep to maintain resistance to parasitic infection, manifested very specifically, as the ability to reject incoming larvae and possibly to expel existing adults burdens, while all other immune responses, *viz.* arrestment of larvae, worm stunting and suppressed worm fecundity were

relaxed. Alternatively, the results may reflect a periparturient relaxation of resistance in all three nutritional groups followed by a subsequent re-establishment of resistance in protein supplemented animals, enabling the expulsion of incoming larvae and possibly existing adult worms. In addition, the results have demonstrated an apparent immune response, in terms of reduced *in utero* egg counts and adult worm burdens, triggered by the post partum challenge infection.

Chapter 7

General Discussion

The factors predisposing sheep to the periparturient breakdown in resistance to GI parasitism were summarised by Barger (1993) as being ascribed to poor nutrition, stress, lack of antigenic stimulation and hormonal suppression of immunity. As yet however, the precise cause has not been determined. Emphasis in attempts to define the cause have recently focussed on investigations of endocrine/immunological interactions - mainly because of the precise timing of the breakdown with the onset of lactation. This thesis appears to be the first to investigate, systematically, the involvement of nutrition in the breakdown.

Nutrition has been shown to be important in the development and maintenance of resistance to parasitic infection in the young animal (Abbott *et al.*, 1985a; Bown *et al.*, 1991; van Houtert *et al.*, 1995). Early workers demonstrated that adult ewes which had previously shown resistance to *H.contortus* infection, could, after a period of undernutrition, revert to a state of susceptibility to reinfection (Clunies-Ross and Gordon, 1933). Later, it was speculated that ewes may indeed experience periods of restricted nutrient supply sufficiently severe to bring about a temporary relaxation of resistance to nematode infection and that this may be manifested as an increase in FECs around the lambing time (Morgan *et al.*, 1951; Wilson *et al.*, 1953; Dunn, 1957). The early studies, however, involved very few animals and nutrient status was not clearly defined. Connan (1971), investigated the role of gross nutrition on the post-parturient rise and found that a breakdown in resistance occurred irrespective of nutritional adequacy. He concluded that immuno-suppression around parturition was unlikely to be primarily due to malnutrition. Little hard

evidence for the involvement of nutrition in the periparturient relaxation of resistance has been presented prior to this study.

In nutrition studies such as this it is important to recognise that results can reflect a number of possible nutritional responses; firstly, the nutrient status of the animal in terms of energy, protein or some other nutrient may have the potential to influence the susceptibility of animals to infection; secondly the degree to which current nutrient intake alleviates the increased nutrient demands associated with late pregnancy and early lactation may be important in maintaining resistance and thirdly, there may well be interaction between either or both of the above and endocrine changes occurring at this time.

In terms of the nutrient status of the animal, LW and CS were recorded throughout the study to assess changes in the energy status of the various groups of ewes and protein status was considered in the final trial by CT estimation of body composition. Neither LW or CS appeared to be particularly well correlated with susceptibility or resistance to parasitic infection. This was most rigorously tested in the first study (Chapter 3) where there appeared to be little effect on parasite status of the animals despite significant differences in LW and CS of 12.3 kg and 0.4 of a CS. In addition, there was no significant effect on nutritional treatment, LW or CS on the animals response to challenge infection at 3 or 6 weeks post partum. Variation in ewe LW and CS of this magnitude are not uncommon in mobs of sheep in New Zealand (Parker and Townsley, 1986).

A number of studies have examined the effect the level of ewe nutrition in late pregnancy, compared with level of nutrition during lactation on lamb weaning weight (Coop, 1950; Monteith, 1971; Rattray and Jagusch, 1978; Johnson *et al.* 1982; Rattray *et al.*, 1982.). While it has been shown that ewe and lamb weaning weights can be affected by the level of nutrition during pregnancy, the effects of nutrition during lactation are far greater (Smeaton *et al.*, 1983; Smeaton and

Rattray, 1984). As a consequence ewes are generally restricted, in terms of feeding prior to lambing, thus saving winter pasture for use during lactation. The obvious problem of metabolic diseases associated with such practices was addressed by Smeaton *et al.* (1985) who concluded that only in cases of severe under-feeding (conceptus-free weights in late pregnancy, 11 kg or more, below mating weight) would this lead to a significant rise in production losses.

Clearly, the scenario of ewes of low LW and CS in late pregnancy may well occur in sheep flocks within New Zealand. The results from Trial 1 suggest that protracted under-nutrition, leading to extreme body weight loss, is unlikely to significantly increase susceptibility to parasitism. These studies did not, however, consider the possible impact of climatic stress, superimposed on chronic undernutrition, since the sheep in the present work were housed. In practice, spells of severe weather, a failure of the animal to eat or an increase in corticosteroids could well be sufficient, in undernourished sheep, to break down resistance.

The level of parasitic challenge has been shown to affect the animals productivity (Brunsdon *et al.*, 1986) and it can be calculated that larval intake around parturition may be considerably higher than at other times of the year, due to the increased feed intake of animals around this time. However, O'Sullivan and Donald (1973) raised the issue of the difficulties of relating pasture intake with larval intake - suggesting that lactating ewes may experience far higher levels of larval challenge because voluntary feed intake may be as much as 50% greater than in pregnant or unmated sheep. Under field conditions it is very difficult to assess the rate of larval intake of individual animals. The majority of infective larvae on pasture (50%) are believed to congregate on the lower 2 cm of the pasture sward (Vlassoff, 1982) but they may also be present in the upper sward in conditions of high humidity and temperature, depending on the plant species (Familton and McAnulty, 1997). This information may have implications for the dynamics of larval intake.

Sheep on a low level of feed intake will graze pasture to a low residual herbage mass, thus potentially being exposed to a greater larval challenge due to the concentration of infective larvae on the lower 2 cm of sward. Conversely, sheep with a higher feed intake, will graze swards of greater pasture mass and theoretically larvae should be more dilute, thus decreasing larval challenge. It could however be argued that the larval intake from the combination of high pasture intake and low larval concentration, could exceed larval intake resulting from low pasture intake of more concentrated larvae. The difficulties involved in estimating rates of larval intake under field conditions have led many workers to design experiments where animals are subjected to a known rate of infection (O'Sullivan and Donald, 1973).

The general awareness among farmers that pregnant ewes need only be fed at maintenance levels after mating (Coop, 1950) and the practice of saving pasture for lactation as discussed above (Smeaton *et al.*, 1985) may therefore have considerable effects on the larval challenge experienced by ewes during pregnancy. The second trial (Chapter 4) was undertaken to determine whether a combination of low nutrient intake and high larval challenge may have predisposed sheep to a breakdown in resistance. Once again, although trends emerged, differences in FECs and worm burdens were not large enough to be statistically significant. Interestingly, from this trial it emerged that the actual burdens consisted mainly of adult worms. This may have indicated that the breakdown - manifested as a failure to reject incoming larvae, had occurred at least three weeks prior to parturition since, by the time the animals were slaughtered, very few juveniles were present, despite infection having continued until the end of the trial.

It was interesting to note from Trial 2 (Chapter 4) that the parasite status of ewes was not significantly affected by the larval challenge of 20,000 *T. circumcincta* day^{-1}. When planning this particular trial, concern was expressed by the Lincoln University Ethics of Experimentation on Animals Committee,

over the high larval dosing regime, proposed for ewes in group L20. Authorisation was granted on the proviso that these animals were to be treated with anthelmintic should their welfare be significantly compromised by the infection. It was anticipated that this group were likely to experience a periparturient breakdown more rapidly and of greater magnitude than the remaining three groups. That this was not the case leads one to speculate as to the level of challenge infection required to break down the immunity of resistant sheep. As outlined in the introduction to Chapter 4, the larval intake of ewes can be calculated to be well in excess of 200,000 day^{-1}.

Bown *et al.* (1991) demonstrated that dietary protein infused directly into the abomasum appeared to enhance the tolerance of young lambs to infection more so than dietary energy, provided in the same way. In recent years, we have acquired a greater understanding of the provision of MP to ruminants, thus enabling greater accuracy in formulating diets. Protein supplementation studies in the past have relied on the CP content of feeds to estimate the amino acid intake of animals. This is likely to be a poor indicator of the actual amino acids which will become available for absorption and metabolism by the animal. In an attempt to address this issue, AFRC (1993) take into account variation in the quantity and the quality of MP as affected by the level of feed intake and subsequent residence time in the rumen. In a review by van Houtert and Sykes (1996) this point was highlighted and examples were given, demonstrating the extent to which MP can vary from CP. In one case, a study by Siddons *et al.* (1985), sheep were fed either silage or hay - the silage providing 59% more CP than the hay. Actual MP provision was estimated to be 10% lower in animals fed silage.

With this as a consideration, Trials 3 and 4, in the present study used the factorial approach of AFRC (1993) to estimate with greater accuracy the supply of MP to ewes. It is recognised that fishmeal, as utilised in the present diets, provides MP of a high quality and this is reflected in the feed composition data

tables of AFRC (1993). The rate of passage of food material is also recognised as varying with feed intake and estimates of this variation were made based on the tables of AFRC (1993). The basis for this assumption is the greater supply of MP at the small intestine with higher rates of digesta throughput occurring with increased levels of feeding as described by Ørskov and McDonald (1979). Other studies have shown that in addition to the level of feed intake, the physiological state of the animal also has an effect on the rate of digesta flow through the GI tract (Gonzalez *et al.*, 1985; Faichney and White, 1988; Weston, 1988). These studies reported that pregnancy and to a lesser extent lactation, were associated with an increased outflow rate of material from the rumen, thus increasing the supply of MP reaching the abomasum and increasing the amount of amino acid available for absorption in the small intestine. At present there are relatively few quantitative data on the degree to which physiological state affects post ruminal digestion of protein from fresh herbage and it does not appear to have been included in the factorial approach of AFRC (1993). For the purpose of the current work, assumptions for diet formulation and MP provision are based only on the information provided by AFRC (1993).

The work by Bown *et al.* (1991) had implied that increased protein supply had a significantly greater benefit compared with increased energy supply in establishing and maintaining resistance to infection. In the third trial in the present study (Chapter 5) this was addressed using fishmeal as a means of supplementing MP. The results demonstrated, quite strikingly, that animals fed fishmeal in late pregnancy and early lactation had significantly lower FECs and worms burdens. Energy supply appeared to have little effect on the animals resistance to infection. In addition the work demonstrated that multiparous ewes appeared to experience a breakdown of resistance of greater magnitude than single bearing/rearing ewes. This finding may strengthen the case that the periparturient breakdown has a nutrition component to it's occurrence. The effect of litter size on the periparturient breakdown has been reported by McSporran and Andrewes (1988), Gruner *et al.* (1992) and Romjali *et al.* (1997).

Gruner *et al.* (1992) hypothesised that the added stress of increased lactation in multiparous ewes, impaired resistance to a greater degree, while McSporran and Andrewes (1988) suggested that multiple bearing ewes may have consumed more infective larvae from pasture than single bearing ewes. The results of Trial 2 tend to question the validity of this statement. Multiple pregnancy will clearly, however, increase protein and energy demand of the animal and the results obtained in Trial 3 (Chapter 5) may well have reflected a response to protein supplementation.

The effect of fishmeal supplementation was confirmed in Chapter 6. In neither of these trials however was it possible to determine whether the effect was in response to protein supply alone or to some specific component of the fishmeal such as a specific essential amino acid. Figures 7.1.1 and 7.1.2 plot MP and CP intake during lactation against worm burdens of twin bearing ewes recorded in Trials 3 and 4. These figures demonstrate the tendency toward reduced worm burden with increasing protein intake. n.b. Figures 7.1.1 and 7.1.2. use arithmetic mean worm burdens so that standard error bars can be plotted.

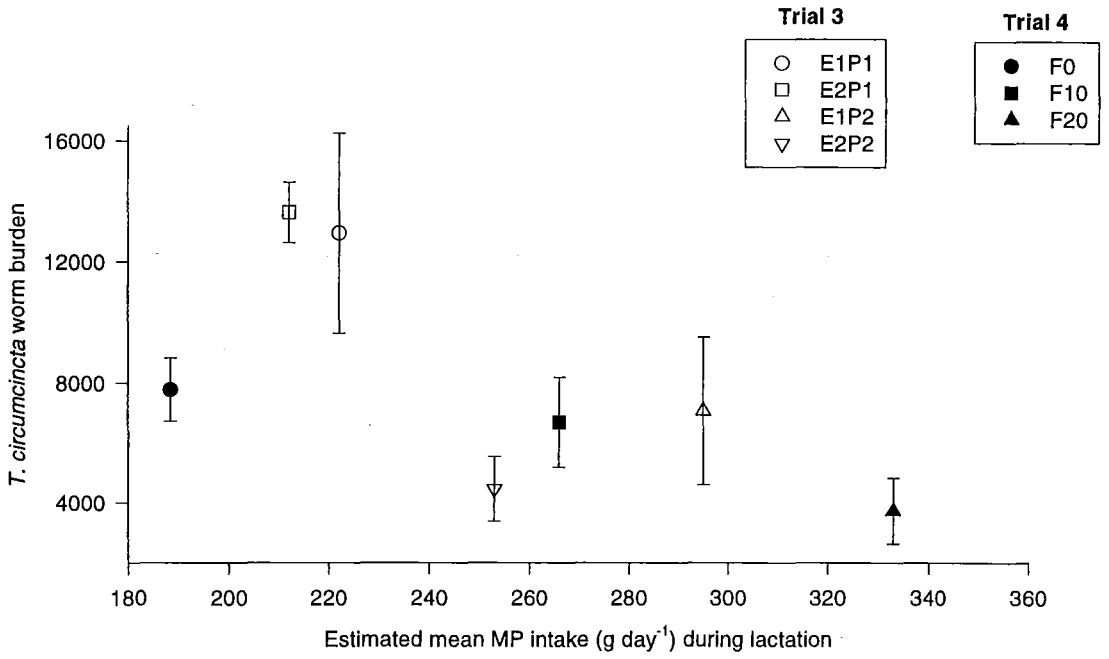

Figure 7.1.1. Plot of *T.cicumcincta* worm burdens (arithmetic mean ± SEM) against estimated mean daily metabolisable protein (MP) intake (g day^{-1}) of twin bearing sheep in Trials 3 and 4

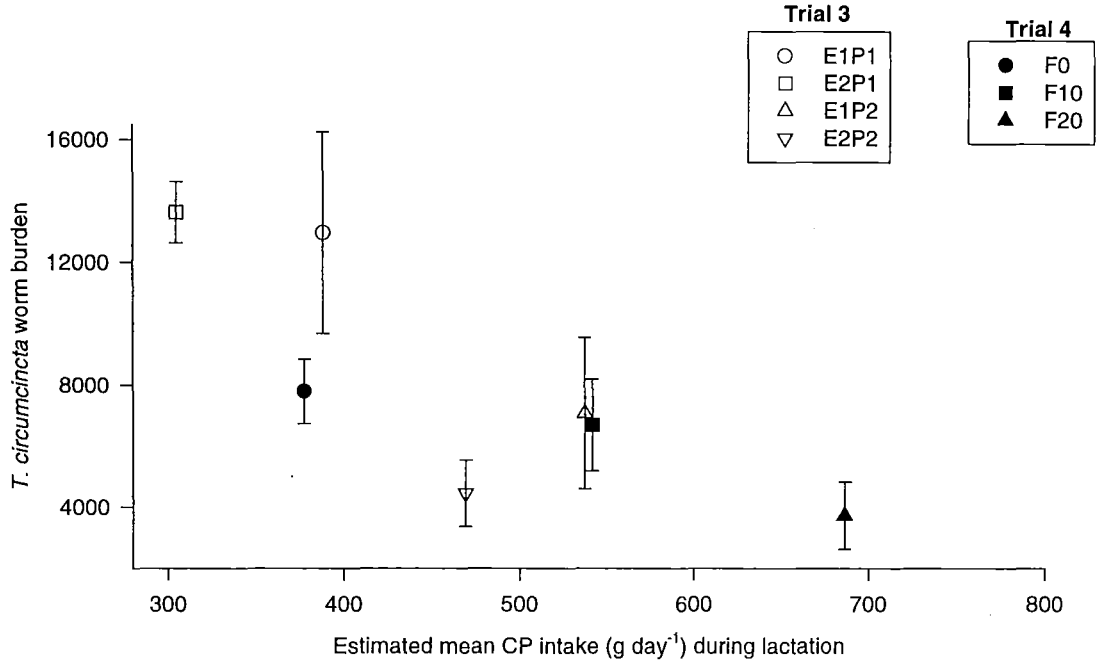

Figure 7.1.2. Plot of *T.cicumcincta* worm burdens (arithmetic mean ± SEM) against estimated mean daily crude protein (CP) intake (g day^{-1}) of twin bearing sheep in Trials 3 and 4

Metabolisable protein intake of P1 ewes in Trial 3 during lactation averaged approximately 220 g day^{-1} which tended to be intermediate between F0 and F10 in Trial 4, where average daily MP intake was estimated to be approximately 190 and 260 g day^{-1}. Despite this, worm burdens were greater in P1 ewes *viz.* approximately 13,000 compared with F0 and F10 ewes *viz.* approximately 7,700 and 6,700 for F0 and F10, respectively. This illustrates the difficulty in comparing responses to protein supplementation year by year as other factors are likely to play a part in the animals susceptibility/resistance to infection. It may well be that both genetic and environmental variation could have influenced the responses observed. For example, climatic conditions prior to housing, may have varied between years and altered the level of larval intake experienced by ewes, thus influencing immune memory. Lamb growth rate and the demands of lactation may also have impacted on the responses of animals between years. However, lamb growth rate between years, appeared to be reasonably consistent (Tables 5.6 and 6.4), although the limited data from Trial 3, precludes any firm conclusions being drawn here. It should also be noted that half of the ewes in Trial 4 had received the post partum challenge infection which, as discussed in Chapter 6, had reduced the numbers of adult worms recovered. Clearly this will have decreased the mean of worm burdens in Trial 4 ewes. In addition, there may have been variation in the infectivity of batches of larvae used between years, but since infectivity was measured only in Trial 4 (Chapter 6) this cannot be confirmed.

The periparturient breakdown may well be triggered by endocrinological changes in the host at this time, which may elicit cell mediated immune responses associated with GI parasitism. MacRae (1993), speculated that these immune responses increase production of leukotrienes, which are rich in cysteine and would therefore increase the demand for S-amino acids. The fishmeal supplementation in the present work and the casein infusion which stimulated increased resistance to *T. colubriformis* in young lambs in the work of Bown *et al.* (1991) may have provided sufficient S-amino acids to meet this

increased demand. Other, non-nitrogenous components may also have been involved. For example, fishmeal, would have increased the supply of polyunsaturated fatty acids. An increase in the availability of arachidonic acid - the precursor of both prostaglandins and leukotrienes - may well have allowed the synthesis of leukotrienes to be maintained at a time of increased prostaglandin synthesis. Mitchell *et al.* (1990) provided evidence of a reduction in the establishment rate of *O. circumcincta* in lambs, in which conversion of arachidonic acid into prostaglandins was blocked with meclofenamic acid.

In the latter two trials (Chapters 5 and 6), the composition of the worm burdens was examined in some detail. The greatest effect of fishmeal supplementation was manifested as a reduction in adult worms present three weeks after parturition. In both cases L4 larval stages were present in low numbers compared with adults, where infection had been terminated three weeks previously (at parturition). This may simply have been a reflection of the three week non infection period being of sufficient duration to enable development of larvae to adulthood. It was of interest to note however, that protein supply appeared to have no effect on inhibition rates. As outlined previously, one of the manifestations of resistance is an inhibition of larval development apparently forcing larvae into an arrested state. One may have anticipated that if protein supplementation was maintaining resistance and preventing a periparturient breakdown, then L4 stages would have been present in greater numbers. Regardless of nutrient status, all animals appeared to have undergone some form of relaxation of resistance to infection.

The experimental design of the third trial was such that it was not possible to determine where the differences in adult worm burdens were occurring *viz.* whether affecting establishment or the animal's ability to expel adult worms. The breakdown of resistance may have occurred in all groups but the rate of regain of resistance may have been enhanced in protein/fishmeal supplemented animals. The incorporation of the post partum challenge

infection in the final trial provided an important insight into the likely effects of protein supplementation, demonstrating that differences in worm burdens were occurring at some point between day one and day eleven of infection. This result tended to suggest that protein supplementation was having an effect on the animals ability to prevent establishment of infection. Such a result is in contrast to the findings of many other workers in the field who have found that in young animals, the protein response generally enables the animal to tolerate infection and subsequently expel adult stages of the parasite rather than to prevent establishment of incoming larvae (Bawden, 1969; Dobson and Bawden, 1974; Abbott *et al.*, 1986; van Houtert *et al.*, 1995). In both situations however (establishment or expulsion of adult worms), the precise effect of supplementation remains unclear. An increased protein supply may enhance the animal's ability to recognise antigenic stimulation. Alternatively, susceptible animals may recognise infection but the ability to mount an immune response may be heavily dependent on protein supply.

Trial three had incorporated an immunological component in an effort to determine possible links between nutrient supply and the mechanisms of parasite rejection. Lymphocyte responses to various mitogens and antigens did not appear to be affected by fishmeal supply. van Houtert *et al.* (1995) had undertaken a similar assay measuring *in vitro* lymphocyte responses of growing lambs fed either 0, 50 or 100 g of fishmeal day^{-1} and infected with *T.colubriformis* larvae. Like the present study, results were equivocal, with no apparent direct link between host nutrition and lymphocyte stimulation. Additionally a larval migration inhibition assay undertaken on small intestinal mucus samples from sheep in Trial 3 also failed to provide evidence of this component of resistance being affected by host nutrition. One could argue that abomasal mucus may have been more appropriate to use in this assay, since the breakdown of resistance looked to have occurred to a greater extent to the abomasal dwelling *T.circumcincta* worms and nutrient responses in terms of changes in the inhibitory characteristics of mucus may have changed accordingly.

These were only two of a number of possible immune mechanisms which may be compromised in sheep during the periparturient period. Others including, local antibody reactions such as gut IgA and local hypersensitivity reactions, including inflammatory mediators such as histamine may be affected by protein supply and may be important in the periparturient breakdown, but were not investigated in the course of this study.

The apparent species specificity of the breakdown has been reported previously (Jackson *et al.*, 1988) and was again evident in Chapters five and six, where a dual infection of *T.circumcincta* and *T.colubriformis* was incorporated. Of the total worms recovered from sheep three weeks after parturition in Trial 3, on average, less than 4% of the burdens consisted of *Trichostrongylus* spp. This tends to suggest that the breakdown in resistance was specific to *Teladorsagia* spp., while for the most part, resistance to *Trichostrogylus* spp. was maintained. It seems likely that this is related to nematode species , rather than to site of infection (abomasum vs small intestine) since other workers (Brunsdon, 1970; O'Sullivan and Donald, 1973; Gibbs and Barger, 1986) have shown similar susceptibility to *O. circumcincta* but not to *H. contortus*, another abomasal dweller - which appears to behave in a similar way to *T. colubriformis*. The differences in species establishment are unlikely to be due to suppression of *T. colubriformis* by *T. circumcincta* since Sykes *et al.* (1988) observed no effect of concurrent infection with *T. circumcincta* and *T.colubriformis* on establishment of the latter. Interestingly, in the present study, despite the low numbers of *Trichostrongylus* spp. recovered, burdens were still significantly affected by pregnancy status and protein supply but not by energy supply.

It is concluded that the periparturient parasite status of sheep can be significantly affected by the incorporation of fishmeal into the diet during late pregnancy and early lactation. Whether this reflected an avoidance of a pregnancy/lactation induced, general protein deficiency or the provision of a

more specific amino acid or non-nitrogenous component required by the animal, to maintain resistance to infection, was not determined. The supplement appeared to affect the rate of establishment of incoming larvae, which contrasts with reports from studies in young sheep where it is the rate of expulsion of adult worms which appears to be affected by increased protein supply. The work also tended to confirm the findings of Bown *et al.* (1991) that resistance to parasitism in more sensitive to protein than to energy provision.

Protein status of the ewe may only be one of a number of factors involved in the periparturient breakdown of resistance to gastrointestinal parasitism. Results from the present study however, suggest that protein supplementation may play an important part in reducing larval contamination of the grazing area. The practical significance of these findings will depend upon the relative costs of providing supplements and the availability of effective anthelmintic compounds. With a greater understanding of the animal's protein metabolism it seems prudent to investigate other protein sources particularly those obtained from forages. Many sheep production systems remain purely pasture based and it is yet to be determined whether similar results can be obtained under grazing conditions. Plants containing condensed tannins, which reduce protein degradation in the rumen, may play an important part, but further research is required in this area.

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APPENDICES

Appendix 2.1. Estimation of larval intake of ewes prior to housed experimental period

Month	Pasture larval count kg^{-1} fresh herbage*	Estimated larval intake day^{-1}**
Jan	369	2214
Feb	170	1020
Mar	271	1626
Apr	874	5244
May	570	3420
June	789	4734

* Based on pasture larval data from Lincoln University Research Farm (McAnulty, Pers Comm.)

** Assuming herbage dry matter intake of 1.2 kg/day and 20% DM

Appendix 3.1 Calculation of estimated daily faecal output of sheep in the four weeks prior to parturition in Trial 1

Method:

Example - Week 3 prior to parturition

DMI (Table 3.1) High plane (HE) - 1090g

Assumed composition of DMI - Concentrate 85% Meadow hay 15%

Therefore HE DMI concentrate 926g
meadow hay 164g

DOM concentrate (Table 3.1) 818g kg DM^{-1} 926 * 0.818 = 758 g DOM

Therefore assume remainder of DMI faeces 926 - 758 = 168g

DOM meadow hay (Table 3.1) 553 g kg DM^{-1} 164 * 0.553 = 91 g DOM

Therefore assume remainder of DMI faeces 164 - 91 = 73g

Total faecal output 168 g + 73 g = 241 g DM

Mean DM % of HE group faeces in week 3 prior to parturition 28%[1]

Total faecal output 861g FM day^{-1}

[1] Refer Appendix 3.1.1 below

Appendix 3.1.1 Mean faecal dry matter (g kg^{-1} DM) of HE and LE sheep in final four weeks of gestation in Trial 1

Weeks prior to parturition	HE	LE
3	280	280
2	340	300
1	300	320
0	270	310

Appendix 3.2 Estimation of larval intake of ewes prior to housed experimental period in all trials

Month	Pasture larval count kg^{-1} fresh herbage[1]	Estimated[2] larval intake day^{-1}
Jan	369	2214
Feb	170	1020
Mar	271	1626
Apr	874	5244
May	570	3420
June	789	4734

[1] Based on pasture larval data from Lincoln University Research Farm (McAnulty, Pers Comm.)

[2] Assuming herbage dry matter intake of 1.2 kg day^{-1} and 20% DM

Appendix 4.1 Calculation of additional dry matter (DM) and metabolisable energy (ME) offered to low plane sheep in response to elevated BHB concentration in Trial 2

Example:

Week 5

Dry matter

A total of 1600g (FM) of additional pellets were offered in this week

At 87.5% DM this equates to 1400 g

On a per day basis this equates to 200 g

Averaged across all 36 low plane animals (L) this equates to an additional 6g DM day^{-1}

Metabolisable energy

Estimated M/D value of pellets 10.2

Therefore additional 6 g DM would provide to 0.06 MJ ME ewe^{-1} day^{-1}

Appendix 4.1.1 Additional DM and ME offered to Low plane sheep in weeks 5 - 1 prior to parturition in Trial 2

Week	Additional DM offered $ewe^{-1}day^{-1}$ (g)	Additional ME offered $ewe^{-1}day^{-1}$ (MJ)
5	6	0.06
4	4	0.04
3	10	0.10
2	4	0.04
1	1	0.01

Appendix 4.2 Calculation of dry matter (DM) and metabolisable energy (ME) of mean daily feed refusals during weeks 6 - 1 prior to parturition in Trial 2

Example:

Week 6

High plane group (n = 12)

Assume average DM % of refusals to be 86 and average M/D 9.0

Total bulked weight of feed refusals for week 290 g fresh weight (FM)

Average dry matter refusals ewe^{-1} day^{-1} = 3 g

Average ME refusal ewe^{-1} day^{-1} 0.03 MJ

Therefore 3g DM and 0.03 MJ ME subtracted from the daily DM and ME allowance to give intake

Low plane group (n=36)

Total bulked weight of feed refusals for week 5880 g fresh weight (FM)

Average dry matter refusals ewe^{-1} day^{-1} = 20 g

Average ME refusal ewe^{-1} day^{-1} 0.2 MJ

Therefore 20g DM and 0.2 MJ ME subtracted from the daily DM and ME allowance to give intake

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Appendix 4.2.1 Mean dry matter (DM) and metabolisable energy (ME) refusal for high plane (H) and low plane (L) sheep in weeks 6 - 1 prior to parturition in Trial 2

Week	Mean DM refusal ewe^{-1}day^{-1} (g)		Mean ME refusal ewe^{-1}day^{-1} (MJ)	
	H	L	H	L
6	3	20	0.03	0.20
5	17	24	0.20	0.20
4	32	20	0.30	0.20
3	35	12	0.30	0.10
2	10	6	0.10	0.05
1	14	4	0.10	0.04

Appendix 5.1.1 Formulation of nutrient requirements during pregnancy and lactation in Trial 3 using regression equations derived from ME and MP requirement tables of AFRC (1993)

Example - Single bearing ewe, zero live weight gain, Week 18 of gestation.

From Table 7.1 (AFRC, 1993)

W (kg)	ME (MJ)	MP (g)
40	8.3	72
50	9.8	81
60	11.2	90
70	12.6	98
80	13.9	107

Plotting W against ME: Regression equation is 2.76 + 0.140 * W

Plotting W against MP: Regression equation is 37.4 + 0.870 * W

Appendix 5.1.2 Regression equations for the calculation of daily ME and MP requirements of single and twin bearing sheep during late pregnancy in Trial 3

Week 16/17	Single	E1	2.44 + 0.125 * LW
		E2	4.94 + 0.125 * LW
	Twin	E1	2.80 + 0.146 * LW
		E2	5.30 + 0.146 * LW
	Single	P1	37.0 + 0.780 * LW
		P2	44.0 + 0.780 * LW
	Twin	P1	39.0 + 0.880 * LW
		P2	46.0 + 0.880 * LW
Week 18/19	Single	E1	2.76 + 0.140 * LW
		E2	5.26 + 0.140 * LW
	Twin	E1	3.28 + 0.172 * LW
		E2	5.78 + 0.172 * LW
	Single	P1	36.1 + 0.870 * LW
		P2	49.1 + 0.870 * LW
	Twin	P1	34.6 + 1.020 * LW
		P2	47.6 + 1.020 * LW
Week 20/21	Single	E1	3.16 + 0.160 * LW
		E2	5.66 + 0.160 * LW
	Twin	E1	3.90 + 0.205 * LW
		E2	6.40 + 0.205 * LW
	Single	P1	40.4 + 0.950 * LW
		P2	53.4 + 0.950 * LW
	Twin	P1	38.0 + 1.170 * LW
		P2	51.0 + 1.170 * LW

Appendix 5.1.3 Regression equations for the calculation of daily ME and MP requirements of single and twin rearing sheep during lactation in Trial 3

Weeks 1- 3	Single	E1	14.4 + 0.095 * LW
		E2	18.2 + 0.095 * LW
	Twin	E1	23.4 + 0.085 * LW
		E2	27.1 + 0.085 * LW
	Single	P1	171 + 0.625 * LW
		P2	184 + 0.625 * LW
	Twin	P1	249 + 0.600 * LW
		P2	261 + 0.600 * LW

Appendix 5.2.1 Calculation of ME content of ration offered to sheep in Trial 3

Example:

Week 16 - Animal 401

Offered

Lucerne hay	690 g FM
Pellet 1 (L1P1)	310 g FM

ME content:

Lucerne hay	690 * 88/100 = 607.2 g DM @ M/D 9.3[1] = 5.6 MJ
Pellet 1	310 * 85/100 = 263.5 g DM @ M/D 14.0[2] = 3.7 MJ

Total daily ME content of ration offered 5.6 + 3.7 = 9.3 MJ

[1] Estimated using the method of Barber *et al.* (1984)
[2] Estimated using the method of Alderman (1985)

Appendix 5.2.2 Calculation of MP content of ration offered to sheep in Trial 3

Lucerne hay	607.2 g DMI * 8.9[1] = 5404.1/1000
Pellet 1	263.5 g DMI * 13.3[1] = 3504.6/1000
Total FME offered	5.4 + 3.5 = 8.9
MCP production	8.9 * 9.6[2] = 85.44
Digestibility of MCP	85.44 * 0.6375[3] = 54.5
UP supply:	
Lucerne hay	607.2 g * 34[4] = 20.6
Pellet 1	263.5 g * 14[4] = 3.7
Total UP offered	20.6 + 3.7 = 24.3 g
Total MP supply	54.5 g + 24.3 g = 78.8 g

[1] Fermentable ME of feed (MJ kg^{-1} DM) from feed composition tablesAFRC (1993)

[2] Microbial protein yield (g MJ^{-1} FME) as function of level of feeding (refer Appendix 5.2.2.1 below) AFRC 1993

[3] Digestibility of metabolisable true protein constant from equation (22) AFRC (1993)

[4] Digestible Undegraded Protein as a function of rumen outflow rate of 0.05/h (mid value of rumen outflow rates of AFRC level of feeding throughout trial)

Appendix 5.2.2.1 Assumed microbial protein yield (g MJ^{-1} FME) during late pregnancy and lactation

Week	16/17	18/19	20/21	Lactn.
MCP yield	9.6	9.8	10.2	10.2

Appendix 5.3.1 Mean crude protein (CP) intake (g day^{-1}) prior to parturition in Trial 3

Group	Week 16 (g)	Week 17 (g)	Week 18 (g)	Week 19 (g)	Week 20 (g)	Week 21 (g)
Single						
E1P1	141.4 ± 2.05	141.8 ± 1.90	131.8 ± 1.90	135.7 ± 2.50	135.7 ± 2.50	170.2 ± 7.17
E1P2	181.3 ± 2.81	183.3 ± 3.44	173.3 ± 3.44	178.2 ± 3.84	176.2 ± 4.42	236.0 ± 8.77
E2P1	124.1 ± 1.64	129.6 ± 0.92	129.6 ± 0.92	119.7 ± 1.87	111.5 ± 0.93	159.4 ± 3.92
E2P2	180.0 ± 2.60	187.0 ± 2.39	177.0 ± 2.39	184.5 ± 2.84	184.5 ± 2.84	236.9 ± 4.18
Twin						
E1P1	156.0 ± 9.90	168.7 ± 6.62	168.7 ± 6.62	166.2 ± 7.78	166.2 ± 7.23	221.8 ± 16.10
E1P2	209.1 ± 3.29	218.2 ± 4.23	218.2 ± 4.23	220.0 ± 6.36	212.2 ± 8.39	313.8 ± 7.23
E2P1	141.4 ± 2.96	153.8 ± 2.61	143.8 ± 2.61	138.6 ± 4.18	137.7 ± 5.70	161.1 ± 11.06
E2P2	209.8 ± 4.33	225.7 ± 5.26	225.7 ± 5.26	223.9 ± 8.88	216.3 ± 4.24	283.0 ± 7.26

Appendix 5.3.2 Mean crude protein (CP) intake (g day^{-1}) during lactation in Trial 3

Group	Week 1 (g)	Week 2 (g)
Single		
E1P1	281.3 ± 3.36	280.7 ± 3.68
E1P2	369.5 ± 2.01	370.2 ± 3.00
E2P1	247.5 ± 1.83	250.1 ± 1.46
E2P2	363.4 ± 2.16	359.6 ± 4.11
Twin		
E1P1	397.2 ± 10.86	379.4 ± 26.58
E1P2	537.2 ± 1.94	537.2 ± 1.94
E2P1	319.4 ± 6.84	289.8 ± 16.15
E2P2	463.3 ± 24.60	475.4 ± 16.67

Appendix 6.1 Regression equations for the calculation od daily ME and MP requirements of sheep during late pregnancy and lactation in Trial 4

ME Requirement	
Week 14/15	1.26 + 0.130 * LW
Week 16/17	0.80 + 0.146 * LW
Week 18/19	1.28 + 0.172 * LW
Week 20/21	1.90 + 0.205 * LW
Lactation	23.4 + 0.0850 * LW
MP Requirement	
Week 14/15	30.6 + 0.800 * LW
Week 16/17	27.0 + 0.880 * LW
Week 18/19	28.6 + 1.020 * LW
Week 20/21	32.0 + 1.170 * LW
Lactation	249.0 + 0.600 * LW

Appendix 6.2 Calculation of MP content of ration offered to sheep in Trial 4

Example: Group F0 Week 18

Meadow hay	374.0 g DMI * 5.9[1] = 2206.6/1000
Pellet 1	780.3 g DMI * 11.3[1] = 8817.4/1000
Total FME offered	2.2 + 8.8 = 11.0
MCP production	11.0 * 9.8[2] = 107.8
Digestibility of MCP	107.8 * 0.6375[3] = 68.72
UP supply:	
Lucerne hay	374.0 g * 7[4] = 2.6
Pellet 1	780.3 g * 28[4] = 21.8
Total UP offered	2.6 + 21.8 = 24.4 g
Total MP supply	68.72 g + 24.4 g = 93.12 g

[1] Fermentable ME of feed (MJ/kgDM) from feed composition tables AFRC (1993)

[2] Microbial protein yield (g/MJ FME) as function of level of feeding (refer Appendix 6.2.1 below) AFRC (1993)

[3] Digestibility of metabolisable true protein constant from equation (22) AFRC (1993)

[4] Digestible Undegraded Protein as a function of rumen outflow rate - 0.35/h for weeks 14 - 17 and 0.05 for weeks 18 - lactation

Appendix 6.2.1 Assumed microbial protein yield (g/MJ FME) during late pregnancy and lactation

Week	16/17	18/19	20/21	Lactn.
MCP yield	9.6	9.8	10.2	10.2

Appendix 6.3 Estimated mean crude protein (CP) (± SEM) offered and intake (g day^{-1}) around parturition in Trial 4

Week	F0		F10		F20	
	Offered	Intake	Offered	Intake	Offered	Intake
Gestation						
16	202.1 ± 4.41	195.1 ± 5.75	263.1 ± 4.81	257.6 ± 6.75	323.2 ± 5.91	323.2 ± 5.91
17	182.3 ± 4.34	171.3 ± 6.06	236.7 ± 4.70	221.6 ± 13.00	291.9 ± 5.65	290.4 ± 5.56
18	220.6 ± 5.18	202.5 ± 6.51	286.3 ± 5.62	271.5 ± 6.07	353.1 ± 6.77	350.9 ± 6.56
19	220.6 ± 5.18	208.3 ± 7.25	286.3 ± 5.62	280.8 ± 7.17	353.1 ± 6.77	351.2 ± 6.48
20	268.8 ± 6.17	243.9 ± 7.59	349.8 ± 6.74	332.5 ± 11.33	430.2 ± 8.00	423.5 ± 6.92
21	268.8 ± 6.17	217.7 ± 6.04	349.8 ± 6.74	296.1 ± 9.77	430.2 ± 8.00	383.4 ± 7.22
Lactation						
1	489.8 ± 2.59	395.0 ± 5.23	644.3 ± 2.79	533.3 ± 16.21	787.2 ± 3.39	672.9 ± 20.98
2	489.8 ± 2.59	365.1 ± 22.65	644.3 ± 2.79	548.3 ± 7.44	787.2 ± 3.39	698.2 ± 7.43
3	489.8 ± 2.59	372.2 ± 23.98	644.3 ± 2.79	543.1 ± 9.01	787.2 ± 3.39	687.9 ± 18.68

Appendix 6.4 Calculation of adjusted CT weight as presented in Tables 6.6 and 6.7

Adjusted tissue[1] weight for T2[2]	$\frac{\text{T2 CT tissue weight}}{\text{CT carcase weight}} \times \text{Actual carcase weight}$
Adjusted tissue weight for T1[3] tissue	$\frac{\text{Adjusted T2 CT tissue}}{\text{Original T2 CT tissue}} \times \text{Original T1 CT}$

[1] Bone, muscle or fat tissue

[2] T2 CT scan undertaken at slaughter

[3] T1 CT scan undertaken nine weeks prior to parturition

Appendix 8.1 Research Publications

Donaldson, J.; van Houtert, M.F.J.; Sykes, A.R. **1998.** The effect of nutrition on the periparturient parasite status of mature sheep. *Animal Science* **67:** 523-533.

Donaldson, J. **1998.** The influence of nutrition on the parasite status of sheep. *Parasite notes* 2nd Edition March 1998. New Zealand sheep Council/Merial New Zealand Limited. pp. 36.

Donaldson, J.; van Houtert, M.F.J.; Sykes, A.R. **1998.** Manifestations of resistance in periparturient sheep given fishmeal supplemented diets. *Annual meeting of the New Zealand Society for Parasitology.* Lincoln University, New Zealand. 3-4 September, 1998. Abstract pp. 16.

Donaldson, J.; van Houtert, M.F.J.; Sykes, A.R. **1997.** The response of periparturient ewes to protein supplementation. *Annual meeting of the New Zealand Society for Parasitology.* Palmerston North, New Zealand. 3-4 September, 1997. Abstract pp. 23

Donaldson, J.; van Houtert, M.F.J.; Sykes, A.R. **1997.** The effect of protein supply on the periparturient parasite status of the mature ewe. *Proceedings of the New Zealand Society of Animal Production* **57:** 186-189.

Donaldson, J. **1997.** The effect of dietary protein on the establishment and maturation of nematode populations in adult sheep. In: G.K. Barrell (Ed.) *Sustainable control of internal parasites of ruminants.* Animal Industries Workshop, Lincoln University , Canterbury, New Zealand. pp. 193-201.

Donaldson, J. **1997.** 'Added Protein Beats the Bug of Parasites'. Farmers Weekly - Sheep Update. 26 September 1997. Reed Business Information. pp. 4.

Donaldson, J.; van Houtert, M.F.J.; McFarlane, R.G.; Sykes, A.R. **1995.** The influence of nutrition on the periparturient parasite status of mature ewes. *Joint meeting of the Australian Society for Parasitology and the New Zealand Society for Parasitology.* Adelaide, Australia. 27-30 September, 1995. Abstracts, pp. 51.

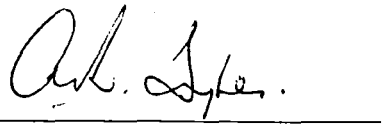
PhD

John Donaldson

This is to certify that John Donaldson has carried out the experiments in his thesis entitled:

"*The effect of nutrition on the periparturient parasite status of sheep*"

under my supervision and in accordance with the course regulations of Lincoln University for the doctoral degree.



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11 August 1998